Proceedings of the 24th Annual Aquatic Toxicity Workshop: October 20-22, 1997, Niagara Falls, Ontario /

Comptes rendus du 24ième atelier annuel sur la toxicité aquatique: du 20 au 22 octobre 1997, Niagara Falls, Ontario

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PREFACE

The 24th Annual Aquatic Toxicity Workshop was held at the Sheraton Fallsview Hotel in Niagara Falls, Ontario, October 20 to 22, 1997. The Workshop included 3 plenary presentations, 129 platform and 49 poster papers. Total attendance was 354.

The 24th Annual Aquatic Toxicity Workshop was one of a continuing series of annual Workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These Workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These Workshops are run by an incorporated National Steering Committee, and the Proceedings are published with the support of the Department of Fisheries and Oceans.

PREFACE

Le 24ième atelier annuel sur la toxicité a eu lieu L'Hôtel Sheraton Fallsview, Niagara Falls, Ontario les 20 au 22 octobre 1997. Le atelier a donné lieu a 3 communications lors de séances plénières, 129 exposés d'invités d'honneur 49 communications par affichage. 354 personnes ont assisté au atelier.

Le 24ième atelier annuel sur la toxicité aquatique a premis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces atelier annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés l'aide du ministère des Pêches et Océans.

EDITORS COMMENTS

This volume contains papers, abstracts or extended abstracts of all presentations at the Workshop. An author index and list of participants are also included. The papers and abstract were subject to limited review by the editors but were not subjected to full formal or external review. In most cases the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned or rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REMARQUES DES EDITEURS

Ces comptes rendus sont publiés en deux volumes, en raison de leur longeur, ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs et une liste des participants sont aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La lonueur et la forme des communications varient parce que ces derniéres sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur le travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elle ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

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PLENARY SESSION/SÉANCE PLÉNIÈRE

ENDOCRINE DISRUPTION IN WILDLIFE SPECIES: RESEARCH AND POLICY CONSIDERATIONS

P.K. Schmieder. U.S. EPA, NHEERL, Mid-Continent Ecology Division, Duluth, MN.

The possibility of chemical contaminants disrupting the endocrine systems of humans and wildlife and, in the latter case, potentially causing adverse ecological effects has recently been the topic of much discussion. The U.S. EPA has sponsored and/or participated in numerous national and international workshops focused on identifying the scope of the problem, developing research strategies to determine potential human and ecological effects, and defining screening approaches to assess the potential for endocrine disruption by the thousands of chemicals regulated by the Agency. This presentation will discuss evidence of endocrine disruption in wildlife species (birds, fish, reptiles, amphibians, and mammals), possible mechanisms for such effects, and research approaches in this area. The talk will also include a researcher's perspective on the process used in developing the U.S. regulatory approach to determining potential for endocrine disruption.

THE NIAGARA RIVER – A TOXIC TALE

D. Draper. The Standard, St. Catharines-Niagara Region, St. Catharines, ON.

What does airplane glue have to do with one of the world's great natural wonders? Airplane glue is exactly what one veteran captain of the Maid-of-the-Mist said the waters below the famed cataract smelled like by the 1960s, after decades of reckless, irresponsible dumping of toxic waste into the Niagara River. Doug Draper, a veteran reporter with The Standard, a daily newspaper in St. Catharines-Niagara, has covered the Niagara River toxics story from the evacuation of hundreds of families around the Love canal dump in the late 1970s to the public protests and efforts by governments on both sides of the Canada-U.S. border to clean the river up. Hold on to your breakfast while Draper takes you on a journey back on hundred years, when the first mega watts of hydro energy were harnessed from the Falls and large industries began lining the shorelines of the Niagara River to take advantage of the cheap power. Almost from the beginning of the modern chemical era that accompanied this boom, the river was used as an industrial sewer. Today, we are still living with the legacy of that abuse. It still isn't safe to eat a prize fish caught downstream from the river, in Lake Ontario, and governments and industries are spending hundreds of millions of dollars cleaning up the sources of the pollution. And it may well be a legacy that we pass on to our children.

20 YEARS ON THE RIVER

F.H. Lickers. The Mohawk Council of Akwesasne, Department of the Environment, Cornwall, ON.

The problems of the environment and society have been with the Mohawk people of Akwesasne since the first Non-Native arrived in North America. The Mohawk's world view has been one of integration with the environment and not domination. Akwesasne is a good example of all the problems which have plagued both Native and Non-Native society. This presentation will concentrate on the different points of view between Native and Non-Native Society with concrete examples supplied by the community of Akwesasne.

PLATFORM PRESENTATIONS/SÉANCE EXPOSÉS

In Vitro Models and Biochemical Indicators

INHIBITION OF PHOTOSYNTHESIS AND GROWTH IN AQUATIC PLANTS EXPOSED TO TRIFLUOROACETIC ACID

C.A. Marwood[1], M. Harris[1], K.R. Solomon[1], D.C.G. Muir[2] and B.M. Greenberg[3]. [1]Centre for Toxicology, University of Guelph, Guelph, ON; [2]National Water Research Institute, Environment Canada, Burlington, ON; [3]Department of Biology, University of Waterloo, Waterloo, ON.

Haloacetic acids are ubiquitous atmospheric contaminants produced by the breakdown of chlorofluorocarbons. Haloacetic acids are persistent, are soluble in water, and have been shown to be phytotoxic. Therefore, these compounds are a potential threat to freshwater plants. As part of a study to validate chlorophyll fluorescence as a bioindicator, the toxicity of trifluoroacetic acid was examined in the rooted aquatic macrophyte *Myriophyllum spicatum* in 12000 L outdoor aquatic microcosms. A single injection was applied at nominal concentrations of 10, 30, 100 and 1000 µg/L. Photosynthetic competence was measured using pulse-modulated chlorophyll fluorescence techniques. Inhibition of photosynthesis was then compared with effects on plant growth at the population-level by harvesting seasonal macrophyte biomass. Exposure to trifluoroacetic acid resulted in concentration-dependent inhibition of chlorophyll fluorescence parameters, indicating reduced photosynthetic rates in *Myriophyllum*. Seasonal plant growth in the microcosms was inhibited in a similar manner. Chlorophyll fluorescence as a bioindicator of trifluoroacetic acid toxicity was well correlated with environmentally relevant population-level endpoints.

WHAT CAN BE LEARNED FROM INVESTIGATIONS INTO THE BIOCHEMICAL MECHANISMS OF TOXICITY? ADVENTURES IN PHOTOSYNTHESIS

B.M. Greenberg[1], T.S. Babu[1], C.L. Duxbury[1], X.-D. Huang[1], A. Mallakin[1], J.B. Marder[2], C.A. Marwood[3] and K.R. Solomon[3]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Department of Agricultural Botany, Hebrew University, Rehovot, Israel; [3]Department of Environmental Biology, University of Guelph, Guelph, ON.

The action of many environmental contaminants involves inhibition of photosynthesis, and thus photosynthetic activity has potential as a bioindicator of contaminant impacts. We are studying inhibition of photosynthesis by PAHs. PAH toxicity is enhanced dramatically by light. A good deal of this increased toxicity is due to photooxidation of the parent PAH to more reactive compounds. The effect of photooxidized anthracene and other oxidized PAHs on photosynthesis was monitored *in vivo* and *in vitro* by measuring chlorophyll a fluorescence, carbon fixation and electron transport. We found that inhibition of photosynthesis was very rapid indicating it is a primary site of action. Comparisons of growth inhibition and impacts on photosynthesis (measured by chlorophyll a fluorescence) were used for laboratory validation of photosynthesis as a bioindicator. The effectiveness of chlorophyll a fluorescence as a bioindicator was further validated at the ecosystem level in a mesocosm study of creosote toxicity. Three fluorescence parameters were consistently found to be predictive of whole organism effects. To understand why inhibition of photosynthesis was an apparent cause of PAH phytotoxicity, the primary site of action of photomodified anthracene was investigated. It was found to be downstream from photosystem II (PSII), at PSI or the cytochrome b/f complex. This was followed by inhibition of PSII and chlorosis, probably due to excitation pressure on PSII as a result of the blocked downstream electron flow. To distinguish between PSI and cytochrome b/f inhibition, PSI

activity was selectively measured *in vivo* following treatment with specific anthracene photooxidation products. The cytochrome b/f complex was identified as the primary site of inhibition. Importantly, the cytochrome b/f complex is almost identical to the mitochondrial cytochrome bc complex (cytochrome c reductase). Thus, bioenergetic function is predicted to be an area of risk in all aerobic organisms exposed to photomodified PAHs.

TOXICANT INTERACTIONS IN COMPLEX PAH MIXTURES

B.J. McConkey, S. Tripuranthakam, Y.S. El-Alawi, D.G. Dixon and B.M. Greenberg. Department of Biology, University of Waterloo, Waterloo, ON.

Polycyclic aromatic hydrocarbons (PAHs) are widespread pollutants, and in general occur in environmental compartments as complex mixtures. However, assessing the toxicity of PAH mixtures based on total PAHs present has not been shown to accurately predict toxicity. The proposed model relates the toxicity of complex PAH mixtures to the toxicity of individual PAHs and PAHs in combination. Toxicity was assessed with a developed screening assay using *Photobacterium phosphoreum*. The assay was conducted under different lighting conditions, allowing the investigation of light dependent toxicity as well as non-photochemical toxicity. Randomly generated combinations of environmentally relevant PAHs were prepared and the toxicity of these mixtures assessed. The generated data was split into two sets, the first set used to assess interactions between toxicants. This was used along with single chemical toxicity data to develop predicted concentration-response curves for PAH mixtures. The second set of the generated mixture data was used to verify the developed model. Further verification of the model was conducted using an environmental sample of PAH contaminated sediment. The sediment was extracted and T.I.E. methodology used to determine the relative contributions of individual mixture components. The observed toxicity of the sediment extract was compared to the predicted toxicity, and found to agree within expected error.

IMMUNOMODULATION CAUSED BY TRIBUTYLTIN (TBT): CAN A BIVALVE STILL RESIST A BACTERIAL CHALLENGE?

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Tributyltin (TBT) is a biocide used as an antifouling agent in marine paints. It is one of the most toxic chemicals ever released into the marine environment. Effects of TBT on the reproductive systems of certain marine invertebrates (i.e., imposex and intersex in gastropod molluscs) are well known but little work has been reported on TBT's effects on the immune system. In this study, we examined immunomodulation in the marine bivalves *Mya arenaria* (soft shelled clam), *Placopecten magellanicus* (giant scallop) and *Mytilus edulis* (blue mussel) as a potential bioindicator of exposure to, and early effects of TBT. Twenty individuals of each species were exposed for 46 d to an environmentally realistic concentration of TBT and 20 individuals were left unexposed as controls. On days 2 and 4, and wk thereafter, two variables were measured from 10 animals per group: [1] numbers of different hemocytes using a Coulter Counter; and [2] phagocytotic activity using a flow cytometer. At the end of the experiment, we challenged the remaining 10 animals to a low concentration of the bacterial pathogen *Listonella angularrium (=vibrio).* In general, the animals from the reference groups of all species successfully fought the infection within one week while conspecifics exposed to TBT could not mount a defense against the bacteria. This result is discussed in relation to the variation in hemocyte numbers and phagocytotic activity.

AN EVALUATION OF THE CYTOTOXICITY AND PHOTOCYTOTOXICITY OF INTACT AND PHOTOMODIFIED CREOSOTE THROUGH THE USE OF A RAINBOW TROUT GILL CELL LINE, TRGILL-W1, AND TWO FLUORESCENT INDICATOR DYES, ALAMAR BLUE AND 5-CARBOXYFLUORESCEIN DIACETATE ACETOXYMETHYLESTE

K. Schirmer, J.S. Herbrick, B.M. Greenberg, D.G. Dixon and N.C. Bols. Department of Biology, University of Waterloo, Waterloo, ON.

The influence of ultraviolet (UV) irradiation on creosote toxicity was investigated with the rainbow trout gill cell line, RTgill-W1, and two indicator dyes: alamar Blue and 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM). Respectively, these monitor metabolic activity and membrane integrity. After solubilization and chemical analysis, creosote was presented to cells in the dark to measure cytotoxicity or concurrently with UV irradiation to evaluate photocytotoxicity. As well, creosote was photomodified by 2 h of UV irradiation prior to presentation to cells in the dark or together with UV. Cytotoxicity was detected only at high nominal creosote concentrations, but photocytoxicity occurred at creosote concentrations 35 fold lower. All the aromatic hydrocarbons in creosote appeared to contribute to cytotoxicity, but photocytotoxicity was due only to the fluoranthene, pyrene, anthracene and benzo[a]anthracene of creosote. Photomodified creosote was much more cytotoxic than intact creosote and this difference was most pronounced in the alamar Blue assay. Likely, this was due to photomodification products that impaired the mitochondrial electron transport chain. Photomodified creosote was slightly less photocytotoxic than intact creosote. Overall these results indicate that UV irradiation potentially enhances the toxicity of creosote to fish in several different but significant ways.

VALIDATION OF THE RAINBOW TROUT HEPATOCYTE MODEL FOR ECOTOXICITY TESTING OF INDUSTRIAL WASTEWATER

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Primary cultures of rainbow trout hepatocyte are proposed as an alternative to the rainbow trout bioassay for evaluating mortality, hepatic metallothionein and cytochrome P4501A activity inductions, and hepatic DNA damage with industrial effluents. The validation study sought to assess the performance of the hepatocyte model (HM) in terms of specificity, predictive value, and concordance. In addition, correlation and artificial neural network analysis were used to model the cell system responses compared with responses obtained in trout. The HM exhibited an overall sensitivity of 90% for detecting the various effects, suggesting that most of the time this method was able to detect effluents that were toxic to trout. The specificity ranged between 68-89%, indicating that hepatocytes were also able to confirm the absence of effects in effluent-exposed trout most of the time, but that in some cases the HM gave false positives, particularly when effects were measured at a concentration greater than 22%. The predictive values showed a similar range (i.e. 67-93%) suggesting that the HM was generally predictive of fish toxicity. The overall concordance ranged between 79-91%, indicating that responses obtained with the HM were consistent with the effects measured in effluent-exposed trout. The lower percentages obtained for specificity and predictive value can be explained by the fact that with some effluents the HM seemed to be more sensitive than the trout assay, since it displayed toxic effects even when none were detected in trout. In effluents that were concordant, a statistically significant linear regression model was derived so that trout toxicity/effect endpoints could be predicted from those obtained with the cell system. This validation study suggests that the rainbow trout hepatocyte model can be used as an alternative testing procedure to the rainbow trout assay. The cell system can be used has a pre-screening tool to distinguish effluents that are likely toxic to fish from those that are not.

ASSESSMENT OF THE NEUTROPHIL RESPONSE ASSAY TO DETECT CONTAMINANT INDUCED IMMUNOTOXICITY IN RAINBOW TROUT

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Neutrophils are specialized cells which have the ability to adhere to glass and other surfaces by the synthesis of special adhesion proteins, when stimulated by pathogenic assault or injury. The nitroblue tetrazolium (NBT) assay measures the incidence of activated neutrophils in the blood of fish exposed to a suspected immunotoxin, compared to levels in unexposed fish. A study was undertaken to evaluate the efficacy of applying this technique to the determination of possible immune responses of rainbow trout (*Oncorhynchus mykiss*) to laboratory exposure to pulp mill effluent. Two aquaria (51 x 27 x 21.5 cm) were stocked each with 13 fingerling fish averaging 8.8 cm fork length in 10 L continuously aerated dechlorinated water held at 15°C±1°C in an environmental chamber. One tank was treated with weak black liquor collected from a northern Ontario pulp mill, to a concentration of 0.035%. Water and the effluent treatment were replaced daily 30 min after feeding. Baseline sampling of peripheral blood was conducted via the dorsal vein, and also at 2, 4, 7, 14 and 28 d. Samples were generally begun within 15 min and with minor modifications, followed the method described by Anderson et al. (1992). Data showed that the concentration of activated neutrophils increased with time to 50% in 2 d, 10 fold in 4 d, and 17 fold in 7 d and 30 times the initial level after 14 d. It stabilized at this point. These findings indicate a direct temporal dosage response; and therefore an immune response. These data are also important because a strong mixed function oxygenase (MFO) response has also been measured in rainbow trout exposed to this effluent. Notwithstanding the need for a few changes to the approach this technique does appear to function as a useful screening tool for measuring likely immune toxicity in fish. However, definitive evaluations would require the application of complementary assays.

CREOSOTE IMMUNOTOXICITY TO RAINBOW TROUT (*Oncorhynchus mykiss*)

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Two consecutive field seasons of immunotoxicity data have been gathered from two independent liquid creosote mesocosm studies. In the first season, rainbow trout were exposed to liquid creosote (0, 5, 9, 17, 31, 56 and 100 μl/L) for 28 d, commencing 103 d after the initial addition of the creosote to the mesocosms. During the second season, rainbow trout were exposed to liquid creosote (0, 3 and 10 μl/L) for 7, 14, 21 and 28 d, commencing 80 d after initial mesocosm dosing. In both cases, immunotoxic response was assessed in terms of pronephros leukocyte phagocytic activity, respiratory burst, peripheral blood or pronephros B lymphocyte marking and plasma lysozyme activity. Pronephros leukocyte phagocytic activity and oxidative burst exhibited a significant dose-response relationship during the first season. Oxidative burst was inhibited, while phagocytic activity was enhanced. The time-course study, while confirming these results, also indicated that peak phagocytic activity occurred around 7 d of exposure, while inhibition of oxidative burst peaked around 21 d. Reduced lysozyme levels and a concentration-dependent reduction in the number of peripheral blood B lymphocytes were also observed in the first study. The results from these studies thus far, indicate that creosote has the potential to alter fish immune response. Polycyclic aromatic hydrocarbons (PAHs), a major constituent of liquid creosote, are the suspected immunoaltering agents.

EFFECT OF CREOSOTE EXPOSURE ON OCULAR DAMAGE AND HEPATIC 7-ETHOXYRESORUFIN-*O*-DEETHYLASE ACTIVITY IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Field studies suggest that cataracts in some feral fish populations are associated with polycyclic aromatic hydrocarbon (PAH) exposure, and *in vitro* tests on cultured fish lenses support these findings. Creosote, a widely used wood preservative composed of 85% PAHs, is a source of contamination in many aquatic systems. In this study, the effect of low level creosote exposure on optical and biochemical parameters in rainbow trout (*Oncorhynchus mykiss*) was examined. Toxicity was evaluated by measuring focal length variability in excised lenses of trout exposed to creosote contaminated mesocosms over a 28 d period. These measurements were compared to hepatic levels of the phase I contaminant metabolizing enzyme, CYP1A, measured as 7-ethoxyresorufin-*O*-deethylase (EROD) activity. Trout from two ponds dosed with 3 μl/L and 10 μl/L creosote had significantly higher focal length variability that fish from the control pond, and lens damage increased with increasing dose. Concentrations of 16 priority pollutant PAHs measured in water sampled from the mesocosms were elevated in ponds dosed with creosote. Ocular damage was associated with increased hepatic EROD activity. This study demonstrated that exposure to creosote resulted in reduced optical quality of trout eyes. In wild fish populations, this effect could lead to detrimental secondary consequences such as impaired feeding and reproductive potential.

CHRONIC RETENE EXPOSURE SUSTAINS MFO INDUCTION IN RAINBOW TROUT

N.M. Fragoso[1], P.V. Hodson[1], J.L. Parrott[2] and M. Hahn[3]. [1]Department of Biology, Queen's University, Kingston, ON; [2]National Water Research Institute, Environment Canada, Burlington, ON; [3]Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, MA.

Induction of mixed function oxygenase (MFO) enzymes in fish is consistently observed downstream of pulp and paper mills. Compounds that cause prolonged induction of MFO in fish are persistent chlorinated organics such as 2,3,7,8-TCDD. However, removal of these compounds from pulp mill waste has not eliminated the toxic effects of effluent on fish. Therefore, sustained MFO induction downstream of pulp mills may be due to more labile compounds, such as retene (an alkyl substituted phenathrene), which typically cause transient induction. Because fish are exposed continuously to pulp mill effluents, we have tested the null hypothesis that continuous exposure of fish to retene does not cause sustained MFO induction. Rainbow trout exposed continuously to retene, a component of pulp mill effluent, showed concentration-dependent increases in hepatic ethoxyresorufin-*O*-deethylase (EROD) activity. EROD activity was sustained over 32 d, but diminished to background levels within 4 d after transfer to clean water. The enzymatic response was confirmed by measuring changes in CYP1A1 protein concentrations, and metabolism and excretion of retene by synchronous fluorometric analysis of bile retene and its metabolites. These data support a role for labile compounds in chronic effects of pulp mill effluents on fish and demonstrate that continuous exposure to labile inducers can sustain MFO induction.

TOXICITY OF RETENE TO EARLY LIFE STAGES OF RAINBOW TROUT

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Exposures of rainbow trout (*Oncorhynchus mykiss*) for 48 d to retene (7-isopropyl-1-methylphenanthrene) from the eyed egg stage to hatch, and from hatch to the onset of feeding caused symptoms of blue sac disease. No symptoms were observed in control fish or fish exposed to acetone, the solvent carrier. Mortality rates were elevated at nominal concentrations in excess of 100 µg/L, and fish that died were severely affected by yolk sac edema, craniofacial deformities, and hemorrhaging. These visible pathologies were evident in fish exposed to nominal concentrations as low as 32 µg/L, the lowest concentration tested. The prevalence of these pathologies increased with retene concentration and they became increasingly evident between hatch and swim-up. In contrast, fin erosion and opercular sloughing were more difficult to observe, and were evident only at swim-up; these symptoms occurred in 100% of all exposed fish and not at all in control fish. These data demonstrate that nominal retene concentrations in water greater than or equal to 32 µg/L caused chronic toxicity to developing stages of trout. Correcting for observed losses of retene from solution during 24 h static renewals, the lowest concentrations causing effects could be as low as 20 µg/L. Because 100% of fish were affected with fin erosion and opercular sloughing, the threshold concentration for effects is likely much lower, and secondary infections could cause increased mortality rates if exposure was prolonged further.

UTILIZATION OF THE HEPG2 (HUMAN HEPATOCELLULAR CARCINOMA) CELL LINE AS AN *IN VITRO* MODEL FOR AQUATIC TOXICITY TESTING

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Both biochemical indicators and *in vitro* models for toxicity testing offer potentially sensitive tools for inclusion into toxicity assessment programs.*In vitro* models must mimic *in vivo* responses to provide meaningful data. Immortal cell lines are available in large quantities from a variety of species, including humans, and are easily maintained in culture making them an obvious choice for consideration as *in vitro* models. However, many immortal cells lines lose their ability to carry out cell specific, tissue-specific functions rendering them less suitable to mimic *in vivo* responses. Carcinoma cell lines, unlike normal cell lines, have been shown to retain some of these lost functions and have become of interest as potential *in vitro* models. The human hepatocellular carcinoma (HepG2) cell line has been well characterized. It produces most of the plasma proteins, has biosynthetic capabilities similar to those of normal hepatocytes, retains cell surface receptors, in serum free culture medium is capable of secreting liver-specific serum proteins, and retains Phase I and II biotransformation reactions essential to the detoxification process. Work in my laboratory has shown that HepG2 produces metallothioneins, exhibits adaptive tolerance to metals, exhibits metabolic responses to dioxin (TCDD) and dibenzofurans (TCDF) that are similar to those found in primary human liver cell cultures, retains estrogen receptors, and produces suitable lipoprotein markers stimulated by estrogenic compounds, indicating that this cell line may be useful in monitoring metal, organic, and hormone disruptor pollutants.

Endocrine Disruptors

REPRODUCTIVE SUCCESS OF JAPANESE MEDAKA (*Oryzias latipes*) EXPOSED TO 4-TERT-OCTYLPHENOL

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There are many chemicals present in the environment which have been identified as endocrine modulating substances, and have the potential to cause significant disruption to the normal sexual development of fish and wildlife. Exposure to the estrogenic compound, 4-tert-octylphenol (4-t-OP), induced the development of testis-ova in male Japanese medaka (*Oryzias latipes*). Testis-ova represent an intersex state where both testicular and ovarian tissue are present in the gonad. It has not been established whether the testicular or ovarian tissue in testis-ova functional. Japanese medaka were exposed to 10, 25, 50 and 100 µg/L 4-t-OP from 1 d post-hatch to 6 mo post-hatch. Reproductive trials were performed to determine whether exposed male medaka were able to fertilize eggs of unexposed females. Female medaka were also assessed to determine whether exposure to an endocrine disruptor affected egg production. Results will be presented on the reproductive potential and success of Japanese medaka after exposure to 4-t-OP.

ENDOCRINE-DISRUPTING POTENTIAL OF FLAVONOIDS IN JAPANESE MEDAKA (*Oryzias latipes*)

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Feral fish downstream of pulp mills experience reproductive dysfunctions probably due to exposure to the presence of endocrine-disrupting chemicals in the effluent. With this study, we are presenting evidence that the flavonoids quercetin and chrysin have the ability to interfere with the normal endocrine/reproductive functions of Japanese medaka (*Oryzias latipes*). After 3 mo of exposure, both flavonoids altered the development of gonads and secondary sex characteristics in medaka. In the chrysin treatments, the presence of ovarian follicles in the testicular tissue of male medaka (i.e. testis-ova), a higher proportion of females in advanced stages of oogenesis (>84%), and a higher incidence of females having fully developed urogenital papillae indicated that chrysin has estrogenic activity. On the other hand, quercetin exhibited androgenic activity by inducing advanced stages of spermatogenesis in all male fish, papillary processes on the anal fin (a typical male secondary sex characteristic) in female medaka, and a high incidence of atretic oocytes in the ovaries of females. There is evidence that chrysin and quercetin are present at high concentrations in the heartwood of trees used by the pulp and paper industry, and therefore, it is possible that flavonoids in general may be responsible for causing some of the adverse effects on reproduction of fish exposed to pulp mill effluents. In addition, medaka have been exposed to the flavonoids apigenin, catechin, galangin, kaempferol, naringenin, flavone, flavonol and flavanone in order to establish structure-activity relationships.

EFFECTS OF ESTROGENIC COMPOUNDS ON THE GONADAL DEVELOPMENT OF JAPANESE MEDAKA (*Oryzias latipes*)

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Several known estrogen agonists, including β-HCH and nonylphenol, have been shown to alter the differentiation and development of the gonad in male Japanese medaka (*Oryzias latipes*). These compounds induce an intersex condition known as 'testis-ova', which is characterized by the presence of both testicular and ovarian tissue in the gonad. However it is not clear whether testis-ova are only induced in medaka by exposure to estrogen agonists. In this study, medaka were exposed from shortly after hatch to 3 mo of age to several concentrations of the estrogen agonists, o,p'-DDT and diethylhexyl- phathalate, and to atrazine, which is known to disrupt reproductive hormones through effects upon the pituitary-gonadal axis. Preliminary data indicate that testis-ova are induced in medaka by exposure to o,p'-DDT and data will be presented on gonadal development in medaka exposed to other test compounds.

PULP MILL EFFLUENT INDUCES CELL SUICIDE IN FISH OVARIAN FOLLICLES

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Exposure of feral fish populations to beached kraft mill effluent (BKME) results in a variety of negative impacts on reproductive fitness including reduced ovarian development, reduced egg size, decreased fecundity with age, delayed sexual maturation and alterations in reproductive endocrine homeostasis at multiple sites among the pituitary gonadal axis. The present study provides evidence of elevated apoptotic DNA fragmentation in ovarian follicular cell from vitellogenic and prespawning white sucker exposed to BKME. Apoptosis is the molecular mechanism responsible for ovarian follicular atresia which involves various stages of vertebrate ovarian development such as follicular recruitment, growth, differentiation, and regression. 3'-endlabelling of isolated ovarian follicular cell DNA revealed up to a 10 fold increase in the extent of apoptosis in BKME-exposed white sucker in comparison to follicles collected from nearby reference sites. The elevated ovarian cell apoptosis in preovulatory fish was associated with reduced ovary size, decreased plasma testosterone and increased plasma 17β-estradiol concentrations, but not induction of hepatic ethoxyresorufin-*O*-deethylase (EROD) activity. By comparison, the increased apoptosis in white sucker undergoing vitellogenic development was associated with reduced plasma testosterone and 17β-estradiol levels as well as elevated EROD activity. Since apoptosis is regulated by several hormone factors and gene products, these data suggest that certain components of BKME increase ovarian cell apoptosis in fish via stimulation of cell death signalling. However, it is unclear whether BKME stimulates ovarian cell apoptosis directly, or if this response occurs secondarily as a result of altered reproductive endocrine homeostasis.

A FATHEAD MINNOW PARTIAL LIFE-CYCLE TEST FOR DETECTION OF WOOD-DERIVED ESTROGENIC SUBSTANCES

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Plant sterols have been detected in the dissolved portion of pulp mill effluent at concentrations from approximately 1-150 μg/L. β-sitosterol is a major plant sterol in effluent that resembles the female hormone 17β-estradiol. It has been hypothesized that the release of β-sitosterol from pulp mills may contribute to adverse impacts on fish reproduction. Juvenile fathead minnows (*Pimephales promelas*) were exposed to either a waterborne plant sterol mixture (approximately 90% β-sitosterol, 8% campesterol, and 2% stigmasterol) or pure estradiol in a partial life-cycle test to determine if plant sterols would alter the onset of secondary sex characteristics or egg production. Graded nominal doses of plant sterols (3-100 μg/L) did not alter fish maturation or cause a significant difference in egg production at these concentrations. At a nominal dose of 250 ng/L, 17β-estradiol caused an evident delay in the onset of secondary sex characteristics in male fish and caused a decrease in egg production. Plant sterols and estradiol were extracted from water samples of fish exposure tanks and quantified using GC-MS. After accounting for method recovery, approximately 60% of the plant sterols and estradiol were recovered. Although plant sterols are present in pulp mill effluent, the concentrations tested here have shown no adverse effects on sexual maturation of fathead minnows. However, the response to estradiol shows the potential of this fathead minnow partial life-cycle test for the detection of estrogenic substances.

ESTROGENIC EFFECT OF COAL-TAR CONTAMINATION ON AN ESTUARIAN POPULATION OF MALE WINTER FLOUNDER (*Pleuronectes americanus*)

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High levels of coal-tar derived sediment contamination has attracted much attention to the Sydney estuary (Nova Scotia) over the past few years. As part of an environmental study of the estuary that has spanned over several years, the response of male winter flounder (*Pleuronectes americanus*) to potentially estrogenic chemicals was investigated. Fish were collected in 1992 and in 1993 over three sampling periods (June, September and October/November) from four stations in the estuary and a control site (St. George's Bay). The stations were selected to reflect a gradient of sediment PAH concentrations ranging from 200 mg/kg (close to the point source) to less than detectable (control site). For each fish, serum estradiol and protein bound phosphorus concentrations (as an indirect measurement of vitellogenin) was quantified. Results show a significant effect of the sampling site on concentrations of protein bound phosphorus in the serum of male winter flounder and no effect on serum estradiol concentrations. A dose-response relationship between the increase of protein bound phosphorus and sediment PAH concentrations was detected. Elevated levels of vitellogenin in the blood of the fish could be the result of an estrogenic stimulus. PAHs have been demonstrated to induce estrogenic responses in male fish after metabolic activation by enzymatic detoxifying systems (e.g. CYP1A family). Although the reproductive success of the fish was not investigated, these results suggest the existence of a potential threat to the flounder population as endocrine disrupting chemicals can inhibit all or part of the reproductive cycle of an organism.

New Methods

ON-LINE TOXICITY MONITORING USING IMMOBILIZED BACTERIA

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There are numerous toxicity tests available for the assessment of effluents from treatment plants. These have frequently been assessed against a broad range of criteria with varying results. However, all these test systems have the disadvantage that they are not suitable for on-line continuous measurement. This means that any changes to plant operation are initially retrospective. Amtox is a new continuous on-line toxicity analyser that can be used for toxicity measurement for protection of effluent treatment plants or by changing the bacterial culture for compliance monitoring on discharge permits. Amtox uses a choice of cultures of nitrifying bacteria. Nitrifying bacteria are highly sensitive to a broad range of toxicants making them ideal subjects for toxicity testing. The pure culture is used for effluent toxicity monitoring and intake protection for portable water and is the most sensitive culture. Typical EC_{50} values for 3.5 dichlorophenol are 2.2 mg/L, this compares favourably with *Daphnia magna* where the EC_{50} 96 h is 8.4 mg/L. Being more sensitive makes the Amtox a suitable surrogate to conventional toxicity tests. The wild culture is grown with some in-built resistance to the presence of common toxicants at low levels and is therefore suitable for use a the front end of effluent treatment plants for the protection of the biological treatment stage. This paper presents the results from a series of Amtox monitors being used in a range of applications including municipal treatment plants, intake protection for portable water and the paper mill industry.

SAMPLE SALINITY ADJUSTMENT FOR CULTURING AND TESTING SEA URCHINS

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Most effluents discharged into the marine receiving environment are initially freshwater in nature. Aquatic toxicity testing using marine organisms requires that such freshwater effluents be prepared and modified for use prior to test exposure. A series of experiments were conducted to compare different treatments of salinity adjustment in order to evaluate toxicity based on sea urchin fertilization success. Commercial dry sea salt (Instant Ocean™) and synthetic hypersaline brine were used to adjust freshwater effluent sample salinity to required levels (i.e., 30 ± 2 PPT). Concurrent sea urchin fertilization tests with effluent samples prepared using the two treatments were conducted and evaluated statistically. No significant differences were observed in effluent toxicity based on these two treatments. However, if salinity-adjusted samples are not allowed to buffer for a minimum of 6 to 12 h, preliminary results indicated that samples are toxic to sea urchin gametes. Based on our experience, and the results of this study, the same buffering period should be applied when preparing synthetic marine water for culturing and testing sea urchins. Results obtained from fertilization assays conducted using freshly-prepared synthetic seawater indicate that such water is toxic to both holding, spawning and testing sea urchins and their gametes. If care is taken and freshly-prepared samples are allowed to buffer for a specified period of time, the two different salinity adjustment techniques yield samples with similar results.

TERMINAL BRANCH HAPLOTPYE ANALYSIS: A NOVEL PHYLOGENETIC APPROACH TO INVESTIGATE GENOTOXIC EFFECTS OF CONTAMINANT EXPOSURE AT POPULATION LEVEL

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Genetic diversity of natural populations consist of historically preserved variation and most recent mutation events. We have developed a phylogenetic approach to identify the haplotypes with recent point mutations, we called terminal branch haplotypes (TBHs), on the mitochondrial DNA (mtDNA) to evaluate genotoxic and evolutionary impacts of contaminant exposure at population level. Using DT-PCR genetyping, a newly developed method in mutation screening, and DNA sequencing, we examined sequence diversity of a 900 bp D-loop region in mtDNA of *Ameiurus nebulosus* from two genotoxically contaminated sites (Hamilton Harbour, ON; Black River, OH) and a pristine location (Old Women Creek, OH). The phylogenetic analysis revealed 5 TBHs in 12 mtDNA haplotypes identified from 49 fish from Hamilton Harbour and 1 TBH in 4 haplotypes from 44 individual from Black River. However, none of the 3 haplotypes found in 47 fish from Old Women Creek was characterized as a TBH. The terminal branch index (TBI, no. of TBHs / sample size) in the two contaminated sites was 10.2% and 2% respectively. Both results were significantly different from the control site (0.0%, OWC). The TBH analysis explored in this study aims to identify newest inheritable mutation events at specific loci in the gene pool of natural populations. We suggest that the TBH can serve as a useful molecular indicator of genotoxic effects due to contaminant exposure both *in situ* and *in vivo*. The elevated incidence of these novel mutagenic haplotypes have profound evolutionary and ecological consequences at population level.

ACOUSTIC HARASSMENT DEVICE USE AT SALMON AQUACULTURE SITES IN THE BAY OF FUNDY, CANADA: NOISE POLLUTION AND POTENTIAL EFFECTS ON MARINE MAMMALS

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The number of salmon aquaculture sites in the Bay of Fundy has increased over the past ten years. An unknown proportion of these sites are using acoustic harassment devices (AHDs) to deter seals from approaching salmon cages. A preliminary survey of AHD use at salmon aquaculture sites in the Quoddy Region and Grand Manan Island, NB, was conducted between August 17 and September 7, 1996. In the Quoddy Region, 46% of aquaculture sites were using some form of AHD during daylight hours. In the Grand Manan area, 22% of aquaculture sites surveyed during the day were using AHDs. An evening survey of four sites in the Quoddy Region revealed that one AHD was activated only during evening hours, indicating our daytime survey data underestimates total AHD use. Three different forms of AHDs (10 kHz signal, 15 kHz signal, and a multi-frequency signal) were identified. The level of noise pollution associated with these devices may negatively impact marine mammal populations, including the threatened harbour porpoise (*Phocoena phocoena*), which use these regions on a seasonal or annual basis.

Wastewater Effects

CONTAMINATION BY PRIMARY DEGRADATION PRODUCTS OF ALKYLPHENOL ETHOXYLATES (APEOs) IN THE DETROIT RIVER AND HAMLITON HARBOUR

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Alkylphenol polyethoxylates are a major class of nonionic surfactants which are microbially degraded in sewage treatment plants (STP) and sediments to more toxic and hydrophobic alkylphenols. Recent data on the estrogenic activity of alkylphenols has prompted interest in the distribution of these compounds in the aquatic environment. In the first component of this study, sediment samples were collected from several sites (n=30) near industrialized and pristine regions of Lake Huron, Lake Erie and Lake Ontario and analyzed for concentrations of 4-nonylphenol (4-NP) and 4-(tert)-octylphenol (4-tOP). Concentrations of 4-NP ranged up to 37 mg/kg while concentrations of 4-tOP ranged up to 23 mg/kg in sediment. These data indicate that 4-NP and 4-tOP are present at mg/kg levels in sediments in proximity to wastewater discharges in urban and industrialized centres. In light of this, sediment samples were collected in a transect from two STP outflows in the Detroit River and two STP outflows from Hamilton Harbour to determine the spatial distribution of 4-NP, 4-tOP and other primary degradation products of NPEOs. Sediment data from these sites indicate that 4-NP and 4-tOP concentrations rapidly decline with distance from the STP outflows. Data will also be presented on the other degradation products of NPEOs (NP1EO, NP2EO, NP1EC and NP2EC).

EFFECTS OF STORMWATER DISCHARGES ON AN URBAN ESTUARY

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The impacts of stormwater discharges were evaluated by conducting sediment quality assessment studies at 36 sites in the Corpus Christi Bay, Texas system, the majority of which were located in proximity to stormwater outfalls. Sediments were analyzed for a wide variety of contaminants and toxicity using solid-phase tests with amphipods (*Ampelisca abdita*) and mysids (*Mysidopsis bahia*), and porewater fertilization and embryological development tests with the sea urchin, *Arbacia punctulata*; microbial analyses and benthic community structure were also enumerated. The majority of the large storm drain sites adjacent to urban areas exhibited significant toxicity in the sea urchin porewater assays but no toxicity was observed with the solid-phase amphipod test. Several of the major storm drain sites had highly elevated concentrations of PAHs and pesticides. Both growth stimulation and inhibition were exhibited at different sites in the growth and survival test with *M. bahia*. Significant alterations in benthic communities based on species diversity and abundance indices, were observed at the more toxic sites. The primary factors responsible for the differences observed were determined using multivariate techniques. The impacts on stormwater discharge sites were also compared with the other sites of concern (e.g., produced water discharges, dredge material disposal, industrial and municipal outfalls) in the study area.

ASPECTS OF TOXICITY AND GENOTOXICITY ASSESSMENT OF HYDROPHOBIC ORGANIC COMPOUNDS IN WASTEWATER

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The objective of the present study is to highlight limitations in wastewater quality evaluation procedures concerning the assessment of the potential (geno)toxic impact of particle-bound hydrophobic organic compounds (HOC's) on receiving ecosystems. To this end, an extensive review of the literature was carried out and major limitations encountered are discussed. One of the most common approaches used in wastewater evaluation is the whole effluent toxicity (WET) technique. If this procedure is of considerable value in that it provides short-term bioassay impact of the raw water effluent sample, WET tests are not designed to account for the (geno)toxicity of persistent chemicals such as particle-bound HOC's. In general, very little work has been done on the particle phase from industrial and/or municipal wastewaters. Some studies, however, have shown that this phase is of considerable importance in the delivery of genotoxins to receiving waters. Further, it has been suggested that such particle-bound HOC's are transported and deposited in receiving waters where they contribute to longer term environmental impact. This, together with the sparsity of work, leads to clear needs for research on improved understanding of the contribution of these particle-bound pollutants from liquid wastes and their specific role in aquatic ecosystems. In conclusion, some recommendations and future research needs to assess potential impact on receiving water of particle-bound HOC's present in effluents are given.

CAUSES OF TOXICITY TO RAINBOW TROUT EXPOSED TO STORMWATER RUNOFF FROM LUMBERMILLS

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Acute toxicity was observed with rainbow trout exposed to stormwater run-off from lumbermills. The primary suspected toxicants were the anti-sapstain chemicals DDAC and IPBC. Both chemicals were evaluated for their individual and joint acute toxicity to rainbow trout. In addition, 'TIE profiles' were determined for both chemicals. However, actual TIEs performed on toxic samples did not conform to the profiles determined for the two chemicals. In fact, metals toxicity was consistently identified in the TIEs and the presence of elevated concentrations of metals in the discharges was confirmed analytically. Moreover, correlations of toxicity and concentrations of DDAC and IPBC from 600 samples collected from over 20 sawmills indicated no relationship between the concentrations of these chemicals and toxicity. Collectively, these data suggest that metals are the primary cause of toxicity in stormwater run-off from these mills and that the bioavailability of IPBC and DDAC under current use patterns and discharge conditions is minimal, at least with respect to acute toxicity.

Small Stream Ecotoxicology

UTILITY OF A CYPRINID AS A SENTINEL MONITOR IN A SMALL STREAM

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It has been demonstrated that a sentinel species-based population evaluation using creek chub (*Semotilus atromaculatus*), a common stream minnow in eastern North America, could provide a framework for initial environmental assessments and focus future research needs in streams. This study compared the results of creek chub species endpoints (e.g. growth condition etc.) analysis to identify whether these responses were manifest in another species with different ecological tolerances and requirements from the same stream habitats. Brassy minnow (*Hybognathus hankinsoni*) is another common stream fish in southern Ontario. Unlike the insectivorous creek chub, the brassy minnow is primarily a detritivore, and is tolerant of turbidity and increased siltation of habitats. This study demonstrated that the brassy minnow is experiencing a similar sentinel species response pattern as identified for the creek chub that encompasses modified growth rates, reduced fecundity, similar condition, and elevated egg atresia. The original diagnosis of the creek chub populations identified either a feeding-habit based niche shift or stress from some unknown sub-lethal factor as the causative force(s) for modified ontogenetic characteristics. Given the brassy minnows tolerances for habitat modifications, the causative force acting similarly on both species must be some type of sub-lethal stressor (e.g. modified thermal regimes, urban run-off). The creek chub may be suffering from both habitat modifications and other stressors as well. Population analysis of a suite of small stream fish with recognized differences in their ecological tolerances and requirements has the power to identify meaningful environmental factors at work in these habitats and help focus future research or remediation activities based on as little as a single season of sampling (spring).

A TRAITS-BASED APPROACH TO DIAGNOSTIC STREAM IMPACT ASSESSMENT USING BENTHIC MACROINVERTEBRATES

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There are thousands of common Canadian benthic macroinvertebrate stream species, and countless studies of land use-induced aquatic impacts. Pattern recognition in land use/species assemblage combinations is daunting, yet necessary for ecosystem management. The need to reduce this variation while retaining ecologically relevant information gave rise to the 'functional approach' to stream impact assessment, where species lists are re-expressed as a conglomerate of traits. The

effects of land uses may similarly be reduced. We have identified five major categories of aquatic environmental alteration: nutrient status, hydrology, toxins, temperature and total suspended solids. We have expanded the traditional traits gamma of nutrient status indicators (functional feeding groups), adding life history, morphological and reproductive characteristics which may aid in diagnosing the remaining four impact categories. Over 2000 common North American macroinvertebrate species have been redefined as a set of their traits we believe are relevant to the five major impact categories. Through a series of ordinations involving species traits and community data from impact sites relationships amongst traits and land uses can be depicted. The relative position of impact and reference site trait suits determines the 'direction' of change of an impact site (in terms of the 5 major areas of impact). Direction of change may be deduced regardless of the state of the reference community, and regardless of confounding effects of multiple land uses. As a preliminary test the usefulness of a traits based approach to impact diagnosis has been demonstrated for a number of industrial impact sites on the Saskatchewan River, Alberta.

Fate and Pathways

ENVIRONMENTAL FATE AND EXPOSURE TO DIETHYLHEXYL PHTHALATE (DEHP)

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ABSTRACT

Diethylhexyl phthalate (DEHP) is a Priority Substances List compound and is used as a plasticizer. In order to identify ways of reducing exposure to humans, a better understanding of the movement of DEHP in the environment must be established. On behalf of Health Canada (note that the views expressed in this paper are not necessarily those of Health Canada), the fate of DEHP in the environment was evaluated, with the aim of quantitatively relating releases during production and use to human exposure (OAEI, 1996). The analysis was performed both deterministically and stochastically. A non-equilibrium, steady-state fugacity model was used to predict the environmental fate of DEHP. Human exposure was then estimated for inhalation of air, and ingestion of food, water, and soil.

The estimated losses of DEHP to air, water, and soil were found to represent a very small fraction of the total amount of DEHP in the environment and, consequently, reducing these emissions would have little or no impact on environmental levels. The overall persistence of DEHP in the environment was also estimated to be low. It was recommended that there be further investigation to ensure that landfills are not a source of release of DEHP to the environment.

Ingestion of dairy products was found to present the largest source of exposure to DEHP for all age groups. Ingestion of beverages (excluding tap water) and foods such as confectionary products are also significant exposure pathways, particularly for adults. It was recommended that measurements of DEHP in the feed of dairy cattle and in milk products be obtained. It was also recommended that alternatives to DEHP for the cap liners used in beverage bottles and in the outer coatings used for confectionary products be sought to reduce these sources of exposure.

INTRODUCTION

Phthalic acid esters (phthalates) are widely used as plasticisers in the production of polyvinyl chloride (PVC) and other plastics. Diethylhexyl phthalate (DEHP), the most commonly used phthalate, was evaluation was conducted to determine whether DEHP was 'toxic', as defined under Section 11 of the Act (Environment Canada and Health Canada, 1994). The report concluded that there is insufficient information to conclude whether DEHP is entering or may enter the environment in a quantity or concentration or under conditions that are having a harmful effect on the environment and that DEHP may enter the environment in a quantity or concentration or under conditions that may constitute a danger. The report also recommended additional monitoring of current concentrations of DEHP and determination of current quantities of DEHP released to the atmosphere.

Numerous review articles have been written on the subject of phthalates and DEHP in particular, but previous reports have not quantitatively related the sources of phthalates with the exposure received by humans. In order to identify ways of reducing exposure to humans, a better understanding of the movement of DEHP in the environment must be established. The overall objective of the study was to review and critically evaluate information relating to the environmental fate of, and human exposure to, DEHP. Specifically, the purpose of the study was to trace the movement of DEHP in the environment from initial release through to human exposure.

METHODS

A search was conducted for information reporting measured concentrations of DEHP in all environmental media: surface water, drinking water, outdoor air, indoor air, soil, sediment, fish, food, vegetation, and sewage sludge. The fate of DEHP in the environment was then evaluated. A unit world-type fugacity model (Mackay and Paterson, 1991) was selected as the most appropriate model for this purpose. The primary advantage of a fugacity model is that the movement of chemical between different environmental media or phases is easily examined since all chemical interactions are expressed in common units, based on fugacity. These chemical flows may include emissions, transfer by diffusive processes such as volatilization, chemical transfer in a flowing medium (such as air or water and termed advection), and chemical loss processes such as reaction.

Fugacity is a thermodynamic concept, related to chemical potential. Equilibrium between two phases occurs when their chemical potentials are equal and, thus, when their fugacities are equal. Fugacity, f (in units of Pa), is linearly related to concentration, C (mol/m^3), through the fugacity capacity, Z (mol/Pa.m^3), such that:

$$C = f Z$$

The fugacity capacity (or Z value) for a phase is a measure of the affinity of a specific chemical for that phase. Thus chemicals will tend to partition into phases which have high Z values. The fugacity capacity is a function of the chemical, the phase, the temperature, and the pressure. Fugacity capacities may be calculated according to the methods described by Mackay and Paterson (1991).

There are several different types of fugacity models, with differing levels of complexity and assumed abilities to simulate the environment. A four-compartment Level III (steady-state, non-equilibrium) fugacity model was selected as most appropriate. The model included exchange of DEHP between the atmosphere, water column, sediment and soil. Each compartment consists of an assembly of subcompartments, which may consist of air, water, solid, and biotic matter. Equilibrium was assumed to apply within each main compartment (i.e. between subcompartments) but not between main compartments. Thus, the air, water, soil, and sediment compartments may have different fugacities (i.e., there may be a net driving force for movement of chemical between these compartments). But, within the water compartment for example, the water and suspended sediment are assumed to be at equilibrium (have equal fugacities).

Mackay and Paterson (1991) suggest non-chemical-specific parameters for the fugacity model for Southern Ontario. Since a large portion of the emissions of DEHP to the environment are in Southern

Ontario, the parameters suggested by Mackay and Paterson (1991) were used. The fugacity calculations were performed using the software Excel™ and Crystal Ball™, which permits stochastic analysis. Traditional deterministic estimates were also calculated. Details of the assumed inputs are provided in OAEI (1996).

The amount of DEHP emitted to each medium (air, water and soil, and lost as solid waste) during the production, use, and disposal of DEHP and DEHP-containing products was estimated. Most of the emissions were based on estimated loss rates since actual measurements were unavailable. By far, the largest mass of DEHP is deposited in landfills, however, emission rates from landfills are believed to be low.

The concentrations predicted using the fugacity model were then compared to measured concentrations. Potential exposure to humans was quantified using measured (or predicted) concentrations in each medium. Exposure was evaluated for the pathways of inhalation of air and ingestion of food and water and incidental ingestion of soil. As a result of the significance of human exposure to DEHP through food consumption (Environment Canada and Health Canada, 1994), the exposure assessment carried out in this study evaluated exposure to 13 separate food groups.

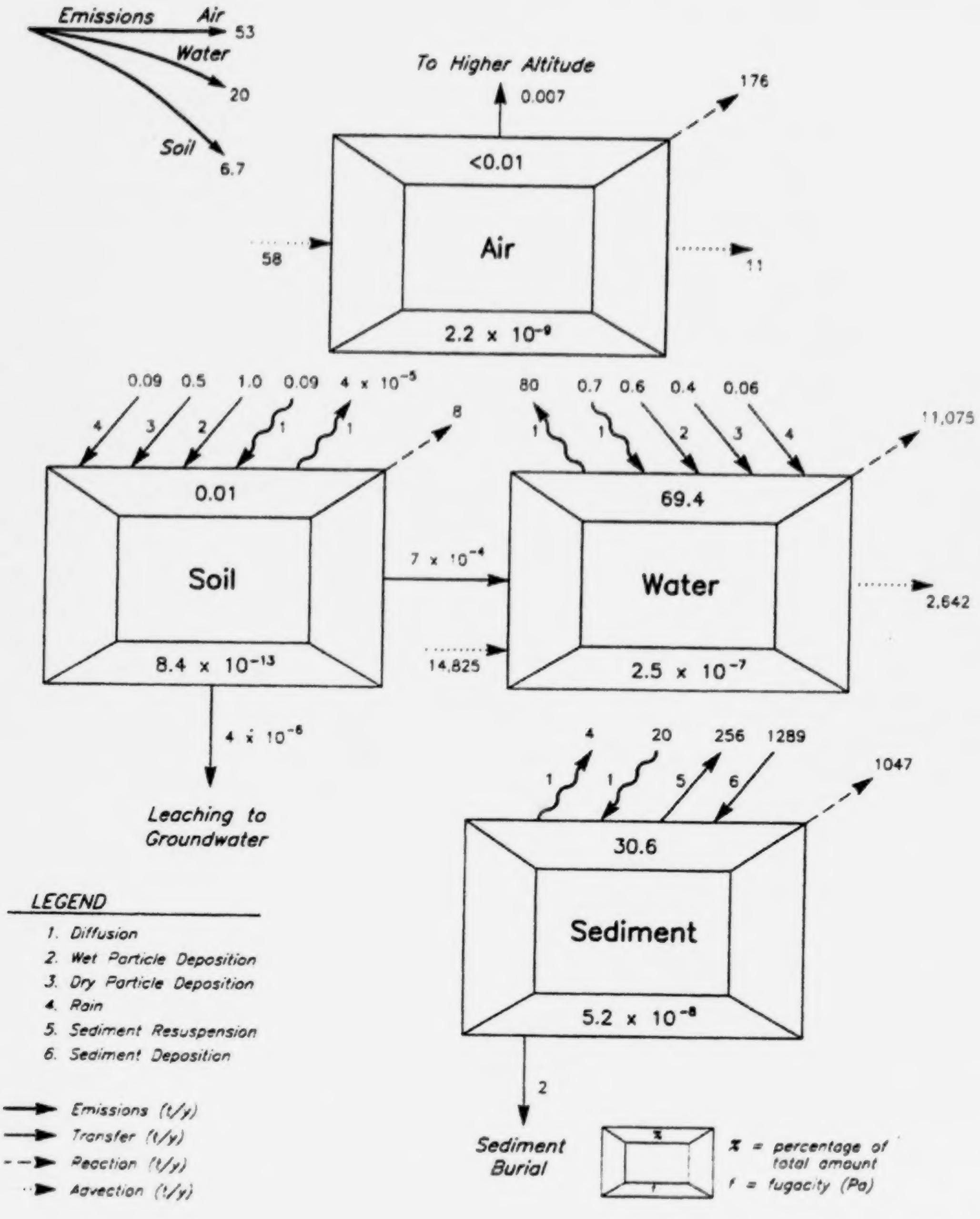
RESULTS

Fig. 1 presents a schematic display of the results of the fugacity modelling, using the deterministic inputs. The rate of transport of DEHP between each compartment (in t/y) is presented, as well as the fugacity and fraction of chemical in each compartment. At steady state, approximately 69.4% of the DEHP is expected to be in the water compartment, 30.6% in the sediment compartment, 0.01% in the soil compartment, and less than 0.01% in the air compartment. For both air and water, the concentration of DEHP leaving the environment by advection in air and in water is less than the concentration which entered by advection.

As shown on Fig. 1, the largest flux of DEHP arises from advection into the environment in water (14 825 t/yr). The water compartment has the highest fugacity, meaning that the net driving force is for removal of DEHP from the water. Approximately 75% of the DEHP entering the water degrades within the water (11 075 t/yr) and 18% leaves through advective flow of water (2642 t/yr). Despite the low rate of reaction of DEHP in sediment, a large quantity of DEHP is reacted in the sediment compartment (1047 t/yr), due to the large mass available for degradation. A significant quantity of DEHP also reacts in the air compartment (176 t/yr). The overall persistence of DEHP in the environment was estimated to be 0.3 yr.

Direct emissions to air, water, and soil represent a very small fraction of the DEHP present in the environment. Although the emissions estimates were based on typical loss factors from various stages of processing and use, and not on actual measured emissions, the emissions would need to be at least 50 to 100 times higher than estimated before they would have a significant impact on the predicted concentrations. Assuming the emission estimates are correct within an order of magnitude, reducing the emissions of DEHP to the environment would have little or no impact on reducing the concentration of DEHP in the different environmental media.

Table 1 presents a summary of the predicted concentrations of DEHP in air, surface water, soil, and sediment. Both the deterministic and stochastic results are presented. The range of measured concentrations is also shown, for comparison. The predicted concentrations for air and water are in general agreement with measured concentrations, although they are at the low end of the measured ranges. The predicted concentration in soil is also at the low end of the measured range. The predicted (deterministic) concentration in sediment exceeds the upper measured concentration by a


Emissions
Air 53
Water 20
Soil 6.7
To Higher Altitude
0.007
176
<0.01
Air
2.2 x 10^{-9}
58
11
0.09 0.5 1.0 0.09 4 x 10^{-5}
4 3 2 1 1
8
0.01
Soil
8.4 x 10^{-13}
7 x 10^{-4}
14,825
80 0.7 0.6 0.4 0.06
1 1 2 3 4
11,075
69.4
Water
2.5 x 10^{-7}
2,642
4 x 10^{-6}
Leaching to Groundwater
4 20 256 1289
1 1 5 6
1047
30.6
Sediment
5.2 x 10^{-8}
2
Sediment Burial
LEGEND
1. Diffusion
2. Wet Particle Deposition
3. Dry Particle Deposition
4. Rain
5. Sediment Resuspension
6. Sediment Deposition
Emissions (t/y)
Transfer (t/y)
Reaction (t/y)
Advection (t/y)
%
f
% = percentage of total amount
f = fugacity (Pa)

Distribution of DEHP in Southern Ontario

Figure 1

factor of 2, although the predicted median concentration is within the typical range. The results of the fugacity model, therefore, appear to be in general agreement with the measured concentrations, although there may be a tendency to over-predict sediment concentrations and under-predict the concentrations in the other media. Analytical difficulties in recovering all the DEHP contained in the sediment may, in part, account for the over-prediction in sediment concentrations. Table 2 presents the percentage of the total daily intake attributed to each exposure pathway, for the five age groups. Ingestion of food represents 99.9% of the estimated intake of DEHP, for all age groups. Ingestion of dairy products contributes most of the intake, ranging from 32.2% in adults to 91.7% in neonates. Ingestion of 'other beverages' (other than tap water) and 'other foods' (including confectionary products) are also significant exposure pathways.

Table 1. Summary of predicted and measured concentrations of DEHP in air, water, soil and sediment.

	COMPARTMENT			
CONCENTRATION	Air (ng/m^3)	Water (µg/L)	Soil (µg/kg)	Sediment (µg/kg)
Deterministic	0.4	0.9	0.02	1400
Stochastic				
Mean	2	1.3	0.06	2300
Mediam	1.4	0.4	0.03	620
Measured	0.5 to 45	<1 to 66	<0.1 to 11	12 to 700

Table 2. Percentage of total daily intake of DEHP via each exposure pathway.

	% of TOTAL DAILY INTAKE		
EXPOSURE PATHWAY	Adult	Toddler	Neonate
Ambient Air	0.0%	0.0%	0.0%
Indoor Air	0.1%	0.1%	0.0%
Drinking water	0.0%	0.0%	0.1%
Ingested soil and dust	0.0%	0.0%	0.0%
Food	99.9%	99.9%	99.9%
Poultry	1.5%	0.9%	0.0%
Fish	1.9%	0.3%	0.1%
Processed meats	1.8%	0.9%	0.0%
Other meats	1.4%	0.5%	0.9%
Eggs	0.0%	0.0%	0.0%
Dairy products	32.2%	66.9%	91.7%
Fruit products	0.3%	0.3%	0.3%
Vegetable producs	8.7%	4.1%	0.8%
Cereals	0.5%	0.8%	1.7%
Grains	5.5%	3.0%	0.2%
Nuts and beans	0.0%	0.0%	0.0%
Fats and oils	2.3%	1.0%	0.1%
Other beverages (excl. water)	28.8%	3.6%	0.1%
Other foods (not listed above)	15.0%	17.6%	4.0%
TOTAL	100.0%	100.0%	100.0%

Very little of the food packaging presently used in Canada contains DEHP, although DEHP is continued to be used in some cap liners, in printing inks on the outside of packages and in the outer coating of packages. DEHP may also be used in the tubing to convey some foods. The source of the DEHP in the dairy products is uncertain. The results of the predictive fate modelling are inconclusive, but do not rule out the possibility of food chain accumulation of DEHP.

CONCLUSIONS AND RECOMMENDATIONS

As a result of the present study, the following conclusions are made: [1] estimated emissions of DEHP to air, water, and soil (using loss factors) represent a very small fraction of the total amount of DEHP in the environment and, consequently, reducing these emissions would have little or no impact on environmental levels of DEHP; [2] the fugacity model was able to adequately estimate the environmental fate of DEHP in the environment. Predicted concentrations of DEHP in air, water, soil, and sediment agreed reasonably well with the measured concentrations. Landfills represent the primary sink for DEHP. Outside of landfills, the majority of the mass of DEHP is predicted to reside in the water column and in the sediment; [3] the overall persistence of DEHP in the environment is estimated to be low. Most of the DEHP enters the environment through advection in water and most of the DEHP is lost through reaction in the water column; [4] ingestion of dairy products presents the largest source of exposure to DEHP for all age groups. It appears that this contamination may be due more to food chain accumulation than due to processing, although this is still highly uncertain; and [5] ingestion of 'other beverages' (excluding tap water) and 'other foods' (such as confectionary products) are also significant exposure pathways, particularly for the adult. Exposure via these pathways could be reduced by discontinuing the use of DEHP in cap liners of beverage bottles and in the outer coatings (including inks) used for confectionary products.

Based on the results of the present study, the following recommendations are made: [1] further investigation should be conducted to ensure that landfills do not present a significant source of human exposure to DEHP; [2] measurements of DEHP in the feed of dairy cattle and in milk products (at different stages of processing, if possible) should be made; and [3] alternatives to DEHP for the cap liners used in beverage bottles and in the outer coatings of confectionary products should be sought to reduce exposure to DEHP.

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BIODEGRADATION OF ASPEN AND CONIFER LEACHATES

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Logyard leachates have been reported to be toxic to aquatic species, however, the environmental fate of these leachates is unknown. We examined the abiotic and biotic degradation potential of these leachates. Leachates produced from trembling aspen (*Populus tremuloides* Michx.) and jack pine (*Pinus banksiana*) were inoculated with bacteria from a receiving stream bed. Dry weight of wood to deionized water ratios of 1:9 and 1:5 for aspen and conifer respectively were used to produce the leachates. Wood was leached for 34 d in the laboratory at room temperature. Initial bioassays showed the leachates to be very toxic to rainbow trout, *Daphnia magna* and Microtox®, with *Daphnia* EC_{50}'s <10% and Microtox® IC_{50}'s <0.3%. Both aspen and conifer leachate successfully detoxified after 20.4 and 23.4 d respectively. Rate constants and half lives were calculated for Microtox toxicity results based on a second-order rate model [Aspen: k= $(2.9\pm7.0)^{10}$, half-life= 370.7 h; Conifer: k= $(3.5\pm1.2)^{10}$, half-life= 490.6 h]. Abiotic factors and physical sorption did not contribute significantly to the degradation of the leachates. GC/MS analyses were performed on the toxic leachates in an attempt to identify the potential toxic components in the samples. The major components in the aspen leachate had a phenolic base. In the conifer leachate, a variety of resin acids were found, along with one terpene and some cresol and phenol.

ENVIRONMENTAL CHEMICAL MODIFICATION IN CONTAMINANT RISK ASSESSMENT

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Structural modification of contaminants in the environment does two things. First, it changes the properties of the compound, in many cases leading to more reactive and hazardous species. Second, the product of the modification reaction is often not part of contaminant load assessments, and therefore opaque to analysis based on the parent compound. This leads to falsely low estimates of environmental chemical loads, and is especially a problem if the modification process leads to more hazardous compounds. A common route of chemical modification in the environmental is photochemical. Photomodification (usually oxidation) of aromatic contaminants is a widespread phenomenon, and generally results in more electrophilic compounds that are hence more reactive. For instance, in the case of PAHs, quinones, phenols and carboxylic acids are generated (oxyPAHs). We have determined the toxicity of several specific photooxidation products of anthracene, phenanthrene and benzo(a)pyrene. Most of these oxyPAHs have been found to be toxic, and indeed many are more toxic than their parent compounds. This elevated toxicity of the photoproducts was observed in *Lemna gibba*, *Daphnia magna* and *Photobacterium phosphoreum*, showing a common phenomenon cutting across biological kingdoms. The mechanism of toxicity of the PAH photoproducts often involved inhibition of electron transport; respiration in animals and photosynthesis in plants. QSAR modelling has been used to describe the phototoxicity of PAHs. The model confirmed the critical role of PAH photomodification in PAH toxicity. We have also begun computer modelling of PAH phototoxicity using highly detailed electron-density shape-fragment maps of PAHs and oxyPAHs. With this chemical shape information, a model is being trained based on the empirical toxicity data for 30 oxy- and intact-PAHs. The trained model should be able to predict the toxicity of a much larger group of related, untested compounds.

PRESENCE, WEATHERING AND BIOAVAILABILITY OF ORGANIC CONTAMINANTS DERIVING FROM RAW SEWAGE AND ENTERING A MARINE HARBOUR

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Nearing the twenty first century, urban populations are increasing at a rapid rate and in many cities around the world, sewer outfalls are dumping untreated effluents directly into marine harbours. Organic, inorganic and organometallic compounds are introduced into the environment through this continuous discharge. Sediments rich in organic carbon content will accumulate the less polar, less water soluble, more lipophilic compounds, such as organochlorine and hydrocarbon priority pollutants. A portion of the chemicals present in these complex mixtures will also be transported on particulates and colloids to further locations than the sewage point sources (Pereira et al., 1996). Weathering which involves biotic and abiotic processes will also take place and this combination of fates has generated the saying that "the answer to pollution is dilution." Biota is exposed to contaminants through the respiration of water and through the diet, while in some cases, organisms can also ingest particulates (e.g. mussels) or larger amounts of sediments (e.g. lobsters).

Table 1. Ratio and concentration (wet weight) of more predominant contaminants in spiked sediments E-0 : 1 : 2 : 5 : 10 : 20 : sludge*

	Ratio	Concentration
Ds and Fs:		(ng/kg)
8D	ND : 2 : 3 : 5 : 8 : 9 : 45	ND : 15 : 22 : 39 : 57 : 66 : 320
PCBs:		(µg/kg)
Aroclor 1260	ND : 1 : 3 : 5 : 8 : 13 : 64	ND : 1 : 3 : 5 : 8 : 13 : 64
Congener 153	ND : 1 : 2 : 2 : 5 : 7 : 35	ND : 0.2 : 0.3 : 0.4: 0.9 : 1.4 : 6.9
PACs:		(µg/kg)
Total	- : 1 : 3 : 3 : 9 : 12 : 66	25 : 95 : 275 : 320 : 850 : 1320 : 6590
C-2NA	- : 1 : 1 : 2 : 3 : 5 : 21	4.0 : 7.9 : 5.3 : 14 : 24 : 39 : 166
Phenanthrene	- : 1 : 2 : 4 : 8 : 12 : 60	1.5 : 5.8 : 8.3 : 22 : 46 : 71 : 360
Pyrene	- : 1 : 2 : 4 : 9 : 14 : 71	0.8 : 6.9 : 14 : 27 : 65 : 100 : 510

*Concentrations determined in E-0 to E-20 are used to deduce the value in harbour sludge (100%). -8D: octachloro-dibenzo-*p*-dioxin, NA: naphthalene.

Table 2. Examples of reported octanol-water partition coefficients and observed bioaccumulation.

Chemical	log K_{ow} Recommended	Time to equilibrium (days)	BSAF* (range)**
Ds and Fs:			
4F	6.1	1007	0.5-1.5
PCBs:			
Aroclor 1260	6.3-7.5	1596-25298	0.5-3
Congener 153	6.9	6355	1-18
PAHs:			
NA	3.4 (3.0 and 4.7)	2	1-7
C-1NA	3.9 (3.7-5.1)	6	0.3-0.8

*BSAF=biota-sediment accumulation factor=C_t / C_s (both in wet weight), similar values would be expected using lipid and organic carbon normalised concentrations.

**If one of the concentrations was non detectable, then no BSAF value was calculated. Values of log K_{ow} obtained from data in Mackay et al. (1991a, b, from Tables 2.2 and 4.2 for recommended values).

The present study focused on exposing winter flounder, *Pseudopleuronectes americanus*, to sediments spiked with various amounts of sewage sludge, for 4 mo during the winter (Table 1). Winter flounder do not feed and have lower respiration rates during the winter compared to the rest of the year, and are also in intimate contact with sediments (Graham, 1988). They can be characterized as dormant during the winter, where exposure to contaminants would be mainly through respiration.

Sludge was collected from St. John's Harbour, in Newfoundland, where raw sewage has been discharged for decades. Winter flounder, *P. americanus,* were then exposed during 4 winter mon to 6 levels of sludge in sediments. A series of organic contaminants, including PAHs, PCBs, DDTs, chlordanes, polychlorinated dibenzo-*p*-dioxins and dibenzofurans were analysed in sediments at the beginning of the experiment and after four mon weathering (Figs. 1 and 2). Concentrations of contaminants detected in spiked sediments were used to deduce the concentration in the original harbour sludge.

Overall, the concentration of PAHs, where alkylated phenanthrenes, fluoranthene and pyrene predominated, were higher than levels of PCBs, while the group of dioxins and furans were present at the lowest concentration. The observed fingerprint indicates a combustion source for the dioxins and a more predominant Aroclor 1260 origin for the PCBs. Although a number of chlorinated pesticides were analyzed in sediments, only p,p'-DDE and p,p'-DDT were detected in the highest exposure (E-20), while traces of some of the other targeted contaminants (e.g. HCB, HCHs, chlordanes) could be detected in sediments. Levels of parental and alkylated PAHs have been examined more extensively throughout the harbour by O'Malley et al. (1996), where PAHs were assigned to combustion and petroleum sources using the molecular and isotopic fingerprint.

Spiked sediments were also analysed at the end of the exposure, where significantly lower levels were observed for the PAHs (range: 1.1-7.1, mean: 3), but not for the organochlorines other than the more predominant dioxins and furans which were present at half the original concentration. Weathering of PAHs was quantifiable, while that of PCBs could not be determined.

Muscle of winter flounder was examined for the bioaccumulation of the same compounds analysed in sediments, to determine the presence of a dose-response. Of the 17 dioxins and furans substituted at C-2, 3, 7, 8; only 2, 3, 7, 8-tertrachlorodibenzofuran could be detected in tissues and at the same concentration in all exposures (0.2-0.3 µg/kg, wet). This could indicate that the environmental level of this furan is relatively similar in the field and exposure media.

A dose-response was observed for the bioaccumulation of PCB congeners 153 and 118 from sediments to muscle (Table 2). More organochlorine compounds were detected in muscle than in sediments. The mean lipid content of muscle was of 0.6%, while the mean wet weight concentration of p,p'-DDE, trans-nonachlor, cis-chlordane, HCB, α-HCH and dieldrin was of 2.0, 1.2, 0.8, 0.6, 0.6 and 0.5 µg/kg. However, no dose-response was observed for these compounds, which is attributed the high log K_{ow} of these compounds which indicates a long time to reach equilibrium within the fish. Compounds with a log K_{ow} higher than 6 have generally been viewed as more bioavailable from the diet, as opposed to those with a lower log K_{ow} than 4, which would be more bioavailable from the water, by uptake through the gills (McKim, 1994).

Of the 17 parental PAHs and C-1 to C-4 alkylated naphthalenes and phenanthrenes, as well as DBT and C-1 and C-2 alkylated derivatives, only naphthalene and C-1 naphthalene were detected, but did not display a dose-response. This would tend to indicate that the lower molecular weight PAHs were present below the threshold level where bioaccumulation was previously observed (Hellou et al., 1994) and that metabolism takes place efficiently at the presently used levels of exposure. The log K_{ow} of the lower molecular weight compounds indicates that these compounds would reach equilibrium within

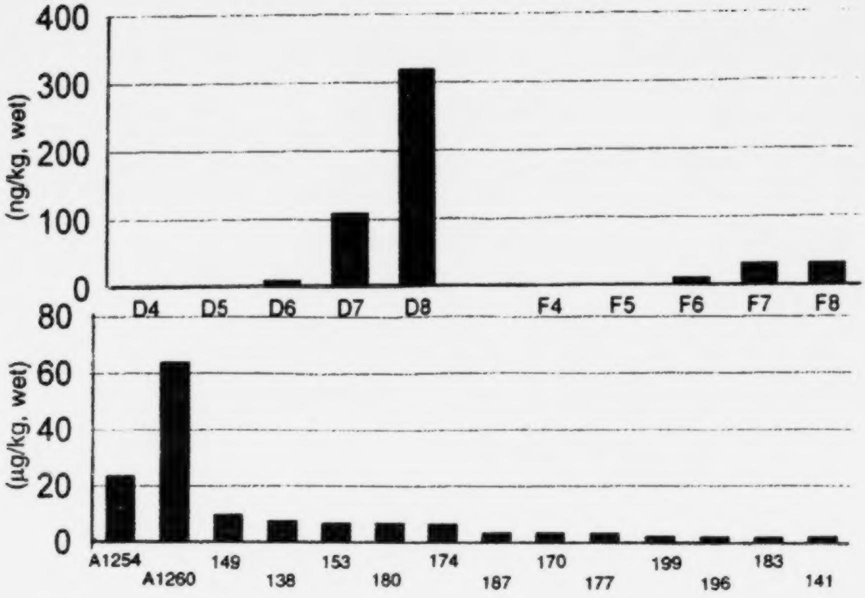

Fig. 1. Organochlorine contaminants in sludge.

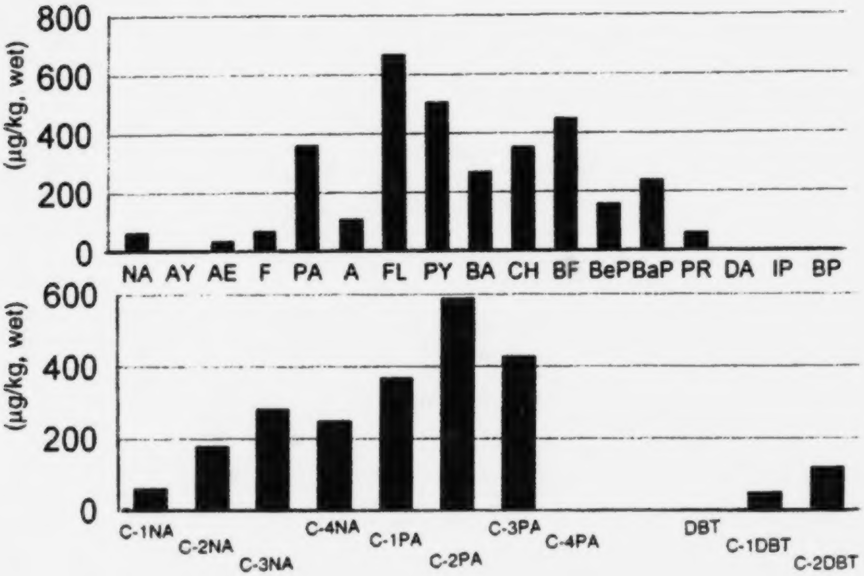

Fig. 2. Polycyclic aromatic compounds in sludge.

fish, during the 4 mo exposure (Mackay et al., 1991a,b). The gall bladder bile of winter flounder was also analysed for bioeliminated contaminants. One bile metabolite, 1-pyrenol, reflecting exposure to combustion PAH was detected at the highest level of exposure, after enzymatic hydrolysis using β-glucuronidase and sulfatase (Hellou and Upshall, 1995).

The concentration of organochlorines is well correlated with the lipid content of tissues (Llorente et al., 1987). Liver generally displays a higher lipid content and therefore would display higher concentrations of OCs than muscle.

This study concentrated on the uptake of contaminants present in sewage sludge, under a specific set of experimental conditions. It demonstrates the presence, bioavailability, uptake of some of the pollutants present in that harbour. Many aspects related to the fate and effect of a variety of contaminants on biota inhabiting coastal areas need pursuing.

ACKNOWLEDGMENTS

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CONCENTRATIONS AND FLUXES OF CONTAMINANTS IN THE ST. LAWRENCE RIVER

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A study was undertaken to measure the levels of contaminants in the St. Lawrence River and to evaluate their fluxes. Water samples were collected over an 18 mo period from 1995 to 1996 using trace sampling techniques. Samples were analysed for metals, PCBs, PAHs, organochlorine and organophosphorus pesticides and triazines in the dissolved and particulate phases. The results show that the levels of contaminants in the St. Lawrence River are very low compared to other water courses. Based on the estimated fluxes, it was found that the export of most of the contaminants from the St. Lawrence River to the estuary is greater than the inputs from Lake Ontario and from the Ottawa River.

Soil/Sediment Assessment

USE OF TOXICITY TESTING IN DEVELOPMENT OF CANADIAN SOIL QUALITY GUIDELINES AND OBJECTIVES

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The Canadian Council of Minister's of the Environment (CCME) has recently released a series of guidance documents to provide a consistent scientific basis for evaluating and managing the risk of contaminants in soil to human health and the environment. The guidance consists of both generic national soil quality guidelines and guidance on setting site-specific objectives for soil quality either through modification of the guidelines and/or site-specific risk assessment. Toxicity testing plays a central role in both the development of generic guidelines and in application of site-specific approaches. General guidance, currently being drafted, is provided on the application of toxicity tests in site-specific assessment and monitoring. The CCME 1996 protocol for derivation of Canadian Soil Quality Guidelines specifies the types and number of toxicity tests required to establish effects-based guidelines for the protection of terrestrial plants and animals, and the ways in which this data will be evaluated in recommending a final guideline value. During evaluation of 20 priority contaminants, significant data gaps were noted in terms of published data on toxicity tests for key terrestrial receptors; and results of standardized toxicity tests for volatile and highly hydrophobic substances indicated that innovative methods may be required to ensure that the effects of these substances in the environment are adequately predicted.

ECOTOXICITY TESTS TO ASSESS BIOREMEDIATION OF SOILS CONTAINING ENERGETIC SUBSTANCES

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TNT (2,4,6-trinitrotoluene), a relatively water soluble nitroaromatic compound, has been identified in soil and groundwater at sites related to military activities worldwide, such as the manufacturing, handling, testing and disposal of explosive and propellant materials. Because of its recalcitrant properties, contamination of TNT in soil represents a significant international environmental problem. TNT is toxic to a number of organisms including humans, and is genotoxic and may be carcinogenic. Studies have been carried out in our laboratory to characterize the ecotoxicity of soils contaminated with energetic substances (such as TNT, RDX and HMX), using pure compounds in solution and in spiked soil samples. This information became the basis for further toxicological studies to monitor the biodegradation of energetic substances in soil at different phases of the bioremediation process. For this purpose, a battery of ecotoxicity tests was used and included aquatic- and soil-based toxicity assays, along with chemical analyses. Bacterial and mammalian cell-based assays were also employed to examine the genotoxicity of some of these compounds. Coupled with chemical procedures for sample preparation and analyses, we demonstrate that a battery of toxicity assays can be used to monitor the toxicokinetics of bioprocesses to degrade energetic substances: a soil slurry bioreactor at laboratory-scale, and a continuously irrigated soil biopile at pilot scale.

BIOLOGICAL TESTING IN ECOLOGICAL AND HUMAN HEALTH RISK ASSESSMENTS

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This talk will focus on the users and providers of biological information for ecological risk assessments. The objective is to demonstrate how biological testing can be incorporated into the risk assessment framework. Biological tests use living organisms as detectors of environmental quality, integrating chemical, physical, and biotic stresses into a response. Responses are generally dose dependent and this provides a measure of the intensity of stress. No assumptions are made a priori with regard to the identities of potential toxic constituents present in complex environmental samples. Criteria based remediation programs rely on lists of target compounds which account for an infinitely small fraction of the total number of known chemicals and potential formulations. From a due diligence perspective, the chemical specific approach can be extraordinarily dangerous. How do you select the most appropriate test method and test species? How do you interpret and apply the results? What are your assurances of data quality? These three main areas will be addressed through a number risk assessments employing biological test data. One important issue is benchmarking a test in order to provide meaningful and comparative data amongst different sites over time. Other issues include sampling, replication, internal controls, positive standards, minimum data requirements, test acceptability criteria, and protocol deviations. A toxicity identification evaluation or TIE can be integrated into the risk assessment as an option for dealing with 'effects.' This technology permits separation, characterization and identification of the stressors. The TIE can be customized to meet study specific data requirements.

THE USE OF *Enchytraeus albidus* AS AN ALTERNATIVE TEST SPECIES TO *Eisenia fetida* IN REPRODUCTION TESTS WITH EARTHWORMS

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Enchytraeus albidus is a small (1 to 3 cm) white pot worm that is found in a variety of field soils in North America. In Europe, it is under investigation as an alternative test species to *Eisenia fetida* in earthworm reproduction tests for new product registration and site assessments. *E. albidus* has the advantage of being ecologically relevant, it is widely distributed and actively moves and feeds in a variety of soil environments. The enchytraeid earthworm is easy to identify, culture, and handle in the laboratory, has a short generation time, and is relatively sensitive to organic contaminants. The use of this species, as compared to *E. fetida*, is of particular interest to risk assessors because of the relative size of the test unit (requires significantly less soil), experimental design (proposed ECx approach), and test duration (shorter generation cycle). As part of a battery of toxicity tests for the assessment of a contaminated site, these advantages could translate into significant monetary savings. *E. albidus* was used to evaluate the toxicity of two contaminated site soils diluted with a clean field-collected reference control soil. Toxicity tests consisted of exposing ten reproductively mature individuals in each test unit to a dilution series (0 to 80 or 100% contamination). Adult mortality was assessed after 14 d (in acute tests) and after 21 d (in reproduction tests as adults were removed from the test soils). Soils for the reproduction tests were further incubated for 21 d, after which, juveniles were enumerated. The organisms were fed and watered weekly, as needed. The results of the preliminary screening toxicity tests and the definitive reproduction tests will be summarized and presented in terms of the sensitivities of the various measurement endpoints (i.e., adult mortality, fecundity, and number of juveniles produced).

THE USE OF AN EARTHWORM AVOIDANCE BEHAVIOUR TEST FOR PREDICTING THE TOXICITY OF CONTAMINATED SOILS TO EARTHWORMS

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A relatively short term (24-72 h) avoidance behaviour test with earthworms was developed with the intention of being able to predict the acute and chronic toxicity of contaminated site soils to earthworms. The duration of this sublethal avoidance tests ranges from 7 to 72 h. The durations of acute and chronic toxicity tests with earthworms are 7-14 d and 28-55 d, respectively. If the results of the avoidance test could be used to predict the acute or chronic toxicity, a very powerful tool would be added to the battery of tests currently available to risk assessors. A circular test unit (O.D. 25.5 cm) was constructed of Plexiglass. It contained six truncated, pie-shaped chambers which surrounded a central circular chamber (I.D. 6.5 cm). Each of the six chambers was connected to the central circular chamber and to adjacent chambers by three arches (1 cm wide, 0.5 cm high). Each chamber could hold about 300 g soil d.w. (400 g soil w.w.). Worms were added to the central chamber and, because of their phototactic response to light, they would quickly move into the chambers with soil. At the end of the exposure duration the location of each worm was recorded. A series of experiments was conducted to examine a number of factors that could potentially affect the results of the test. The results of these experiments will be briefly summarized with emphasis placed on the relationship between the results o the avoidance tests and the acute and chronic toxicity tests.

THE BACTERIAL EXOENZYME TEST: VALIDATION OF A STANDARD OPERATING PROCEDURE

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Numerous polymeric organic compounds are too large to penetrate the cytoplasmic barrier. For their mineralization, bacteria must first excrete exoenzymes to hydrolyze these compounds to facilitate intracellular uptake. Considering the ecological significance of this mechanism on the recycling of organic matter in the ecosystem, we have developed a sediment bioassay procedure based on the inhibition of exoenzyme activity within 'uncontaminated reference' sediments following exposure to 'contaminated test' sediments. Assessments of the developed bacterial exoenzyme test procedure with accepted regulatory bioassays (e.g., Microtox®, echinoids fertilization, ToxiChromo Pad®) showed its sensitivity to both toxic metals and organic pollutants. An external validation of the Standard Operating Procedure (SOP) was conducted with an artificial pollution gradient, prepared by diluting contaminated sediment in clean diluent sediment. These test sediments were also assessed with the Microtox Solid-Phase Test, the Amphipod Survival Test, and the Echinoid Fertilization Test on sediment porewater. Results showed that bacterial exoenzyme test was highly reproducible and more sensitive in pollution detection than the echinoid fertilization and amphipod survival.

A MICRO-ALGAL SOLID PHASE TEST TO ASSESS THE TOXIC POTENTIAL OF FRESHWATER SEDIMENTS

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Phytoplanktonic species play crucial functional and structural roles in aquatic ecosystems and can be adversely affected by sediment resuspension via both natural (e.g., flood scouring) and man-made (e.g., dredging) activities. To assess the degree of toxicity of freshwater sediments which may arise from both readily available and ad(ab)sorbed contaminants, we have developed a 'direct contact' solid phase assay with the widely-used Chlorophyte *Selenastrum capricornutum*. With our procedure, algal cells are exposed for 24 h to serial dilutions of test sediments. The capacity of exposed cell esterases to cleave the non polar stain fluorescein diacetate (FDA) to liberate a polar and fluorescent by-product fluorescein will then determine the extent to which the algae have been intoxicated by the sediment. Individual cell fluorescence (from fluorescein) can then be rapidly and precisely quantified with the help of flow cytometry to determine a toxicity endpoint which relates to esterase inhibition and cell membrane integrity. Thus far, this direct contact sediment phytotoxicity assay was appraised with certified reference sediment materials prepared by the National Water Research Institute in Burlington as well as with some naturally contaminated sediments originating rom various locations in the Québec portion of the Saint-Lawrence River. Toxicity data generated for these sediments with the direct contact assay will be presented and discussed.

FACTORS INFLUENCING ESTIMATES OF SEDIMENT TOXICITY USING THE MICROTOX□ SOLID PHASE TEST: INSIGHTS GAINED FROM STUDIES ON HALIFAX HARBOUR AND BAY OF FUNDY SEDIMENTS

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The Microtox® Solid Phase Test (SPT) is used extensively to describe toxicity of sediments, yet a quantitative understanding of key variables affecting EC_{50}s has been largely ignored. We are paramatizing the influence of known variables, particularly accounting for bacterial loss onto particles as a function of particle concentration, particle size, water content, organic carbon (OC) and quality of particle surface available for bacterial interaction. Halifax Harbour studies showed that the 25 min EC_{50} was associated with OC and silt sized sediments, both of which were highly correlated to metals. One control sediment showed unexpectedly high toxicity, likely a false positive (Type One Error). Studies on Bay of Fundy sediments focused on the relationship between location, particle size, OC, water content and EC_{50}s. Sediments were of low toxicity (5,000-15,000 mg/L); EC_{50}s correlated with location on beach, particle size and OC content. Novel microbiological experiments addressed bacterial loss during the SPT to particles at the filtration stage. Experiments compared toxicity of raw sediments, autoclaved sediments (axenic particles) and autoclaved/washed sediments (axenic, stripped particles). Bacterial loss and sediment concentration were positively correlated for pooled, autoclaved and washed Bay of Fundy sediments, but no significant correlation was generally found for autoclaved-only sediments. Autoclaved and washed sediments were more toxic than autoclaved-only sediments, possibly reflecting increased exposure of test bacteria to particle surfaces. The study points to some fundamental considerations when developing and applying microscale sediment bioassay techniques.

ASSESSING SPATIAL EXTENT OF IMPACTED AREAS IN THE ST. CLAIR RIVER USING THE SEDIMENT QUALITY TRIAD

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The Lambton Industrial Society has commissioned a multi-year study to determine the spatial extent of degraded sediments in the St. Clair River as previously defined by the Ontario Ministry of the Environment. Tools used to assess the status of the sediments included, sediment physical chemistry, sediment toxicity tests (*Hexagenia* spp., *Pimephales promelas, Chironomus riparius* and *Tubifex tubifex*) and benthic macroinvertebrate community structure. Attention was focussed on three zones between Sarnia and Stag Island that had been previously defined as Priority 1 by the St. Clair River remedial action plan. Of 28 Priority 1 stations sampled, 17 were not distinguishable from the 3 reference sites on the basis of benthic macroinvertebrate community structure. Bulk sediment chemistry analyses showed that all stations including reference stations contained at least one constituent that exceeded the Provincial Sediment Quality Guideline (PSQG) Lower Effects Levels while hexachlorobenzene exceeded the PSQG Severe Effect Level at 8 stations and mercury at 11. Toxicity tests showed acute or sub-lethal effects at 27 of 31 stations with reference stations exhibiting toxicity to at least one species. Integration of the three tools used to assess the sediments suggests that 7 of the 31 sites exhibit pollution-induced degradation. A comparison of these results with past studies shows that degraded zones are shrinking spatially.

METHODS FOR INTEGRATION OF ST. CLAIR RIVER SEDIMENT QUALITY TRIAD DATA

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From 1994 through 1996 work has been completed, on behalf of the Lambton Industrial Society, to determine the spatial extent of sediments considered degraded within the St. Clair River. This work has focussed on three small zones of depositional sediments located along the Ontario side of the river, adjacent to the main areas of industrial activity. The tools used for assessment of the sediments included determination of sediment chemistry, sediment laboratory toxicity tests using 4 aquatic species with 7 endpoint responses including endpoints which assess the test organism's energetics (i.e. growth and reproduction) as well as assessment of the benthic community that must be compiled, analyzed, integrated and presented. As part of this work three methods, with varying levels of data complexity, were utilized that provided a method of data integration/presentation. These methods of data integration included; [1] the use of the traditional positive (+) and negative (-) assessment approach but with the inclusion of a half plus "-" for specific data scenarios; [2] development of a colour coded pie chart which integrates each component of the sediment triad; and [3] development of a numerical sediment quality index based on results of the colour coded pie chart. The methods of data integration/presentation provide a mechanism by which environmental data can be synthesized into a format that can be readily used for presentation of complex environmental monitoring data to local community groups and the general public.

WHERE TO FROM HERE? FORMULATING REMEDIATION STRATEGIES WITH EVOLVING DATA

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The St. Clair River has long been the subject of intense, well-documented scientific scrutiny. Contaminant levels, and the health of biota, in both the water column and sediment have been tracked for more than 40 years. Studies proceed chronologically from clear evidence of degraded water and sediment quality to clear evidence of remarkable recovery. Remediation needed to complete that recovery centres on improving municipal discharges and combined sewer overflows, and managing three small areas of impaired sediment where assessment studies are continuing. Formulating a strategy to complete the river's restoration is a task that has been assigned to local communities, through the Remedial Action Plan program. This paper identifies efforts to interpret and apply data from complex studies to develop a sediment management strategy to complete restoration of ecosystem health. Historical studies are shown to provide essential context within which to build a responsible management plan.

COMPREHENSIVE COMPARISON - POREWATER VERSUS SOLID PHASE TESTS

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Recent comprehensive sediment quality assessment surveys along the Pacific, Atlantic and Gulf coasts of the U.S. in which a suite of toxicity tests have been conducted synoptically has afforded the opportunity to compare the relative sensitivity and performance of the different tests. More than twenty estuaries totalling more than 1,000 sites have been sampled and have been tested for toxicity using the sea urchin porewater fertilization test and the amphipod 10 d solid-phase test on split subsamples from these sites. Specific examples from this data set will be presented to illustrate the relative sensitivity of these tests and the concordance between predicted adverse effects, based on sediment quality guidelines, and the observed toxicity will be compared (Long et al., 1995; MacDonald et al., 1996). For example, 73% of the 165 undiluted porewater samples in Tampa Bay, Florida, were significantly toxic with a wide range of responses as compared with only 2% for the amphipod test (Carr et al., 1996). There were highly significant associations between the concentration of a variety of contaminants and toxicity observed in the porewater tests but not in the amphipod tests and a high degree of concordance between the predicted and observed toxicity for the porewater tests but not for the amphipods (Long et al., 1995; MacDonald et al., 1996).

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Long, E.R., D.D. MacDonald, S.L. Smith and F.D. Calder. 1995. Incidence of adverse biological effects with ranges of chemical concentrations in marine and estuarine sediments. Environ. Manage. 19: 81-97.

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ENVIRONMENT CANADA'S NEW REFERENCE METHOD FOR MEASURING SEDIMENT TOXICITY USING MARINE OR ESTUARINE AMPHIPODS

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During 1997, Environment Canada developed and standardized a new Reference Method for determining the acute (10 d) lethality of samples of dredged material or contaminated sediment to marine or estuarine amphipods. This Reference Method follows and is built upon its companion, multi-purpose biological test method 'Acute Test for Sediment Toxicity Using Marine or Estuarine Amphipods', which was published in 1992 by Environment Canada and is soon to be amended. The new Reference Method is one of the biological test methods to be used as part of sediment assessments consistent with Federal Ocean Dumping Regulations under Part VI of the Canadian Environmental Protection Act. It is to be applied using one or more of the following species of amphipod crustaceans: *Rhepoxynius abronius, Eohaustorius washingtonianus, Eohaustorius estuarius*, and *Amphiporeia virginiana*. Included in the Reference Method are species-specific appendices which provide guidance on the known tolerance limits of each species to salinity, coarse and fine-grained sediments, ammonia, hydrogen sulphide, and a reference toxicant. Species-specific application limits for test materials are also defined, which identify the acceptable ranges for certain variables (e.g., porewater salinity and grain size) that must be met if the test material is to be evaluated for toxicity using a particular species of estuarine or marine amphipod. This new test method provides: [1] criteria which must be met for a valid test; [2] criteria for judging whether a sample of test material is toxic or not; and [3] minimum reporting requirements.

THE ASSESSMENT THE THREE SEDIMENT BIOASSAYS TO PREDICT ADVERSE BIOLOGICAL EFFECTS FROM METAL CONTAMINATION OF SEDIMENT

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The Aquatic Effects Technology Evaluation Program (AETE), a government-industry partnership, is investigating several cost-effective technologies to assess mining-related effects in the aquatic environment. These technologies could in turn be used in a national Environmental Effects Monitoring (EEM) program tailored to the particular requirements of the mining industry. The assessment of three sediment toxicity methods was part of the first pilot study, conducted in the Val d'Or region of Quebec, in 1995. The toxicity test battery was composed of the Microtox® solid phase test, the survival and growth of *Hyalella azteca*, and the survival and reproduction of *Tubifex tubifex*. Sediment samples were collected at fourteen stations located in the vicinity of mining sites and tested with the battery. Sediment chemistry and physical properties as well as benthic macroinvertebrate community assessment were used to assess the sensitivity of the three assays and their ability to predict adverse biological effects related to the presence of metals in the sediments. The practicality and cost-effectiveness of each assay were also evaluated. The results obtained indicated that the three laboratory assays responded slightly differently to contaminated sediments when compared to impacted areas as determined by the benthic community structure. Furthermore, the complimentary features of the assays emphasized the usefulness of a test battery for sediment toxicity assessment.

ASSESSMENT AND EVALUATION OF THE EFFECTS OF PARTICLE SIZE, AMMONIA AND SULFIDE ON THE 10 DAY AMPHIPOD SEDIMENT ACUTE LETHALITY TEST

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Concerns have been raised recently that sediment particle size, ammonia and sulfide can alter the apparent toxicity of sediments in the amphipod lethality test. To study the role of these factors as causative agents for measured toxicity, the Environment Canada Atlantic Regional Laboratory used several commercially available sand and clay samples, mixtures of these samples, as well as natural sediments. Ammonia, sulfide, and a marine sediment reference sample of PAHs (NRC reference HS-3) were spiked into these sand and clay mixtures. Four estuarine/marine amphipod species were used as test organisms: *Amphiporeia virginiana, Eohaustorius estuarius, E. washingtonianus* and *Rhepoxynius abronius.* Preliminary results of this study will be discussed to evaluate the interrelated relationships between particle size, 'natural contaminants' (i.e. ammonia and sulfide), and sediment chemical contaminants (i.e. PAHs) and their effects on the amphipod bioassay end-points.

WHOLE SEDIMENT TOXICITY TESTS AND THEIR APPLICATION IN DETERMINING THE RELATIVE SENSITIVITY OF 4 BENTHIC INVERTEBRATES TO DIFFERENT CLASSES OF COMPOUNDS IN SPIKED AND NATURAL SEDIMENTS

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Whole sediment toxicity is presently assessed by Environment Canada (EC) with the following tests: *Chironomus riparius* 10 d growth and survival test; *Hexagenia* spp. 21 d growth and survival test; *Hyalella azteca* 14 d growth and survival test; and *Tubifex tubifex* 28 d survival and reproduction test. Two of the four tests (*C. riparius* and *H. azteca*) are standardized EC protocols. The relative sensitivities of these four invertebrates to different classes of compounds is currently being assessed by performing these sediment toxicity tests with a variety of compounds spiked into a clean reference sediment, as well as by performing sediment toxicity tests with natural contaminated sediments. For spiking purposes, the toxicant is added directly to the sediment and stirred on a side to side shaker for 90 min. Contaminant concentrations were measured in the bulk sediment, pore water and overlying water fractions. To date only Cd results are available. *Hyalella* is the most sensitive organism to Cd in spiked sediment with a survival LC_{50} in spiked sediment ranging from 16.3-22.6 mg/kg Cd, followed by *C. riparius*, *Tubifex tubifex* and *Hexagenia spp.* However, when comparing growth, *Hexagenia* is the most sensitive of the species, with an IC_{25} ranging from 3.1-4.4 mg/kg Cd, followed by *H. azteca*, and *C. riparius*. The IC_{25} values for *T. tubifex* reproductive endpoints were much higher than those reported for endpoints of the other species, with an IC_{25} of 192.5 mg/kg Cd for the number of young produced per individual worm, and an IC_{25} of 430.1 mg/kg Cd for the number of cocoons produced per individual worm.

NON-CONTAMINANT EFFECTS TESTING AND REFINEMENT OF CULTURE CONDITIONS USING TWO SPECIES OF SPIONID POLYCHAETES

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Over the past four years, Environment Canada has been developing a chronic sublethal sediment toxicity test for survival and growth using Canadian species of marine or estuarine polychaetes. The first phase of the project involved the selection and rating of potential test species from the Atlantic, Pacific, and Arctic coasts of Canada. The second phase involved culturing trials with five species chosen for further study, and it was found that two Spionid polychaetes, *Polydora cornuta* and *Boccardia proboscidea*, could be cultured over multiple generations in the lab. Based on these results, a draft Environment Canada test method using Spionid polychaetes was prepared and distributed for review. The review committee identified the following areas which required further work: [1] improvement of culturing techniques; [2] defining the effects of temperature (20 or 23°C) and salinity (5, 10, 15 and 28 PPT) on culture and test performance; [3] further definition of the effects of sediment grain size and organic content; and [4] definition of the tolerance of each species to ammonia. The work is in progress and the results will be discussed in the context of test conditions and data interpretation, thought to be appropriate for Environment Canada's test method using Spionid polychaetes.

Petroleum/PAHs

AQUATIC AND TERRESTRIAL TOXICITY ANALYSES OF DRILLING WASTES – A CASE STUDY

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Drilling waste is a complex matrix known to contain hydrocarbons, drilling lubricants, mud additives, etc. Regulations are in place in Alberta governing the land disposal of the drilling wastes. One of the tests required under the regulations on land disposal is the Microtox® test on the waste. The methodologies for conducting Microtox testing on soil samples will be presented along with other soil toxicity tests using seed germination and root elongation, earthworm lethality and microbial inhibition. The soil toxicity tests are required to assess the quality of the soil after application and to ascertain its ability to support invertebrates, vegetation, and microbial populations. Data from a case study will be presented.

DEVELOPMENT OF MEDAKA EMBRYO-TOXICITY BIOASSAYS TO ASSESS SURFACE WATERS ASSOCIATED WITH OIL SANDS MINING WASTE DISPOSAL

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The oil-sand mining industry is currently developing environmentally acceptable tailings disposal methods as part of their land reclamation strategy. Fine-tailings, a suspension of clay and residual bitumen, contains naphthenic acids, a family of naturally occurring surfactants in bitumen. Naphthenates are released into water as sodium salts during oil-sand extraction processes and have been identified as the acutely toxic component of fine-tailings. The acutely lethal effects of naphthenates are known to decrease over time, possibly due to biodegradation. The 'wet-landscape' method involves capping fine-tailings with a layer of surface water. It is expected that over time a selfsustaining ecosystem will be established. Experimental pits of different ages and fine-tailings/natural water compositions were constructed to assess the potential of this waste disposal method. Whole water and water extracts from these pits were tested for embryo-toxicity using a bioassay with Japanese medaka. Water extracts were further fractionated into acid (containing naphthenates) and base-neutral fractions (containing PAHs) to determine the causative agent(s) of embryo lethality. Results were compared to toxicity tests conducted with concentrations of a sodium naphthenate standard ranging from 80 to 0.625 mg/L to determine embryo-toxic concentrations for naphthenic acids of increasing age. Medaka bioassay results were also compared to laboratory and field experiments using yellow perch to examine the biological relevance of the bioassay. Monitoring naphthenate toxicity with time will allow a better understanding of biotic processes occurring in water-capped tailings ponds and prediction of subsequent risks to the reproductive success of exposed fish.

LABORATORY GROWTH ASSESSMENT OF FATHEAD MINNOW IN WATER AFFECTED BY OIL-SAND PROCESSING

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Waste products, produced by Syncrude Canada Ltd. during extraction of bitumen from oil sands, include tailings water (TPW) and Fine Tailings (FT). Toxicity Identification Evaluation (T.I.E.), indicate the acute fractions of both TPW and interstitial waters associated with FT consist primarily of organic acids, specifically napthenic acids. Past studies have demonstrated that the acute toxicity of TPW and FT decreases over time. The objective of this test was to assess growth of >24 h old fathead minnows (*Pimephales promelas*) exposed waters from Syncrude test ponds. 65 newly hatched fathead minnows were introduced into 18 L aquaria resulting in 4 replicates of seven treatments consisting of waters from test ponds of differing ages and TPW/FT concentrations. Survival was assessed on a weekly basis. Water volume and food were standardized and adjustments for differences in fish density were also made weekly. At 7, 28 and 56 d, a subsample of minnows from each tank was measured, weighed and sacrificed for dry weights. Results of this bioassay were not significant across treatments for survival, length, wet weight or dry weight ($p = 0.05$). These results differs from those of preliminary standard 7 d fathead minnow larval growth and survival tests performed on the same waters which indicated significant differences in growth and survival.

AQUATIC TOXICITY ASSOCIATED WITH DRILLING FLUIDS

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Drilling fluid management and disposal in Alberta is based upon observations made following testing using the *Photobacterium phosphoreum* assay (Microtox®). At present, chemical additives are applied to commercial drilling fluids to enhance performance. Examples include butyl phosphates and Zn carbonates. The addition of these additives raises the question as to the relevance of the Microtox assay to screen drilling fluids for toxicity prior to disposal to the receiving environment. In this study, a battery of aquatic tests including: *P. phosphoreum*, Microtox, bacteria, *Daphnia magna*, invertebrates, *Selenastrum capricornutum*, algae and *Pimephales promelas*, fathead minnow, fish, was applied to assess the aquatic toxicity associated with drilling fluids. A toxicity identification and evaluation was performed in order to isolate and identify the causative agent(s) of toxicity. NOELs and LOELs were determined using Microtox, the larval growth and survival test using *P. promelas* and the reproduction and survival test using *Ceriodaphnia dubia*. Observations concerning the relevance of using Microtox for aquatic protection and drilling fluid waste management will be presented.

CHRONIC TOXICITY OF THE 'WATER SOLUBLE FRACTION' OF NORMAN WELLS CRUDE OIL TO JUVENILE FISH

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Young rainbow trout were exposed to Norman Wells crude oil for periods as long as 55 d using a flow through system. Mortality was light for the first few days but it continued throughout exposure with more rapid and increased mortality at the higher exposure levels. The mortality was generally exacerbated by the presence of oil dispersants Corexit 7664 or 9600. Fish surviving the 55 d experiment showed severe fin erosion and apparent 'flooding' since mean body water content was increased from about 84% to over 90%. We hypothesize that the oil affected the ability of the fish to regulate their water content since the presence or absence of the oil dispersants did not seem to deter this effect from occurring.

BIOLOGICAL RELEVANCE AND INTERPRETATION OF POPULATION, HISTOLOGICAL AND BIOCHEMICAL PARAMETERS IN YELLOW PERCH - AN OIL SANDS EXAMPLE

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A plethora of biological endpoints have been devised elucidate patterns of stressors on fish exposed to anthropogenic compounds. Often, the difficult question of biological relevance is ignored during environmental studies. In order to address some of the relevance of commonly measured biological endpoints, yellow perch were stocked from a natural lake into experimental ponds. Ponds contained either a bottom consisting of fine tailings, or, were situated in clay and/or lean oil-sands. Chemical stressors in the ponds included naphthenic acids, polycyclic aromatic hydrocarbons (PAHs) and high sulphate salinity. Perch were stocked immediately post-spawning and subsamples were sacrificed at 5 mo and 11 mo in order to measure age structure, physiological indicators of energy storage and energy utilization, liver MFO activity, bile PAH equivalents, plasma steroid hormone levels and gill and kidney histopathology. Patterns observed in perch physiological indicators showed improved energy storage and utilization in the as compared to perch in the lake from which the stocked fish originated. This was indicated by increased gonad size, condition factor, liver size and the disappearance of spawning periodicity. Perch from experimental ponds showed moderately elevated MFO enzyme activity, increased PAH bile fluorescence, and an increase in gill lesions, but no differences in levels of plasma steroid hormones. Evidence of decreased disease resistance corresponded with elevated PAHs and increased salinity. Despite biochemical and histopathological responses measured, no indication of reproductive impairment was observed. Thus the biochemical and histopathological indicators are not likely to indicate reduced population integrity unless they impact directly upon fish survival.

INDIVIDUAL- AND POPULATION-LEVEL EFFECTS OF LIQUID CREOSOTE ON FATHEAD MINNOWS (*Pimephales promelas*) IN MICROCOSMS

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A microcosm study was performed to identify the toxic effects of creosote on the fish species *Pimephales promelas*. Two different contamination scenarios were tested. A 'press dose' was applied as a semi-continuous influx simulating a creosote leachate, while a 'pulse dose' was applied independently as a single event like a creosote spill. Mortality, reproductive activity and juvenile survival and biomass were monitored as individual- and population-level assessment endpoints. Hepatic EROD-activity during a time-exposure experiment was examined to check for uptake of creosote. Creosote appeared to be lethal when applied as a 'press dose' even in the lower concentrations (0.3-30 µl/L), while reproduction occurred only in the 0.1 µl/L treatment at a much reduced capacity relative to controls. In the 'pulse dose' series creosote seemed to be lethal at concentrations greater than or equal to 1 µl/L. Juvenile survival was decreased in the 0.1 µl/L treated 'press'-microcosm compared to the control, but juvenile biomass was increased probably due to food abundance. EROD-induction could be measured in both treatment series although the dose response was more distinct in the 'pulse dose'. In both 'press' and 'pulse' treatments, time and concentration were significant variables influencing EROD-induction. Creosote influenced abiotic and biotic ecosystem elements negatively so that the fish were also stressed from secondary factors such as increasing temperature, low concentrations of dissolved oxygen and decreased food abundance through low zooplankton abundance. Creosote appeared to be highly toxic to fathead minnow populations at environmentally relevant concentrations.

AVIAN IMMUNE FUNCTION STUDIES ON RECLAIMED WETLANDS ON OIL SANDS MINING SITES

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Wild, nestling tree swallows (*Tachycineta bicolor*) and semi-captive, juvenile, mallard ducks (*Anas platyrhynchos*) inhabiting reclaimed wetlands or natural wetlands receiving run off from consolidated tailings ponds on oil sands mining sites in Fort McMurray, AB, are part of an ongoing study to determine the ecological viability of these areas. Immunological, reproductive and dietary variables were studied in tree swallows, while in waterfowl, we examined immune response, growth rate, hematology, biochemistry and pathology. Immunological tests included *in vivo* tests for cell mediated immunity (T lymphocyte proliferative response), and antibody mediated immunity. The nonspecific immune response was examined through *in vitro* phagocytosis by peripheral blood mononuclear cells (PBMC). Early results have shown a decreased T-lymphocyte responsiveness to intradermal mitogen challenge in both species. Decreased immune function in 'exposed' mallard ducks was expressed as increased morbidity and mortality during the study period, with respect to control ducks. This study has been designed to complement 'on site' studies in lower animal and plant communities, providing a means of detecting subtle and early contaminant related changes in vertebrates at higher trophic levels living on reclaimed, rehabilitated wetlands on large scale oil sands mining sites.

Metal Effects

CYTOSOLIC METAL SPECIATION STUDIES BY SEQUENTIAL, ON-LINE, SE-IE/HPLC-ICP-MS

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A procedure involving directly coupled HPLC-ICP-MS is described for the quantification of metals associated with cytosolic proteins. Selectivity is achieved by sequential fractionation by size exclusion (TSK SW2000) followed by ion exchange chromatography (Showdex DEAE). Spectra on up to 11 masses for 6 elements was acquired simultaneously by scanning the quadrapole in the peak hopping mode. A flow injection loop inserted downstream of the columns was used to monitor analyte recovery and quantify the ion intensity profiles from the MS. Reproducibility of analysis was approximately 2-5% and absolute detection limits were typically between 10-60 pg of analyte. The utility of the technique for: [1] detecting abnormal distributions in cytosolic metals due to metal exposure; and [2] determining the biological turnover of Cu and other metals associated with proteins is demonstrated using the marine shellfish *Littorina littorea* exposed to elevated concentrations of Cd and ^{65}Cu.

MACROINVERTEBRATE COMMUNITY RESPONSE TO SEDIMENT TOXICITY IN LABORATORY MESOCOSMS

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Small aquatic mesocosms, containing naturally co-adapted communities, exhibit typical ecosystem properties or processes. Preliminary studies established that intact box cores of lake sediment can be brought back from the field and maintained under laboratory conditions, with little change in the resident benthic fauna. The following studies were designed to address the changes in community assemblage as a result of sediment contamination. Replicate boxes were spiked with clean sieved sediment contaminated with copper, cadmium, or an organically enriched solution. A further set of boxes were left unspiked to act as a control. Cluster analysis and ordination were employed in the analyses of community composition and its response to these toxicants. Multiple Discriminant Analysis was then used to relate the community responses to the environmental variables recorded. The results are presented in the form of ordination plots. Changes in the community assemblage of the boxes respond predictably to these stresses, and can be addressed further with respect to the direction in which the ordination plot diverges from its original state.

BIOAVAILABILITY OF CADMIUM TO BENTHIC INVERTEBRATES: CONSTRUCTED WETLANDS FOR STORM WATER TREATMENT

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This review describes the toxicological effects which are associated with the transference of Cd from constructed wetland sediments to benthic invertebrates. Based on existing literature, it may be said that constructed wetland sediments can act as both a sink and a source of Cd. It may also be said that benthic invertebrates will readily accumulate Cd which is then available for uptake by higher organisms. Although Cd does not appear to biomagnify, ecological imbalances can occur as the result of lethal impacts which eliminate organisms at the base of the food chain. Such impacts can be prevented through the implementation of comprehensive monitoring programs and mitigative measures.

TOXICITY TESTING FOR MANGANESE USING FRESH WATER AND MARINE SPECIES

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Concerns have been expressed by regulators about high levels of metals found at several contaminated sites in British Columbia. For some of these metals such as manganese, appropriate water quality criteria/guidelines to assess impact of the contaminated sites on the aquatic environments are not available from Canadian jurisdictions. The U.S. Environmental Protection Agency's water quality criteria for manganese are dated and its validity has been questioned by those required to remediate contaminated sites. For these reasons, BC Ministry of Environment began to develop an aquatic toxicity data base in 1995, using species found in British Columbia, to assess the impact of these types of metals for both fresh (three hardnesses) and marine waters. Species tested include rainbow trout, coho and chinook salmon, *Daphnia magna* (acute and chronic), *Hyalella azteca*, chironomid tentans, purple sea urchins (egg fertilization), early life stage salmonid, Microtox®, amphipods (*E. wash* sp.), and *Deandaster excentricus* (sand dollars). The results of these tests for manganese show that for acute toxicity, toxicity decreases with increased water hardness, but this relationship varies with species. For marine water, chinook and *E. wash* sp. had similar toxicity results, and toxicity was considerably lower than for fresh water. The species most sensitive at each water hardness seems to vary.

THE EFFECTS OF URANIUM IN LAKE WHITEFISH (*Coregonus clupeaformis*) EXPOSED VIA THE DIET

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As U mining and milling activities cause U enrichment of aquatic systems, it is relevant to determine potential effects on fish. Adult lake whitefish were fed a commercial diet contaminated with three concentrations of U, 100 mg U/kg, 1000 mg U/kg, and 10000 mg U/kg, for 10, 30, and 100 d. Whole organism morphometrics and metallothionein content of liver and kidney were unaltered by U exposure. Hematological variables were either unchanged or only transiently affected. Concentrations of serum lipid peroxides were significantly elevated in all treatment groups on days 30 and 100, indicating that U damages cellular and sub-cellular membranes. Significant histopathologies, observed in both liver and kidney, displayed dose- and duration-dependent increases in frequency and severity. Most consistent and pronounced lesions of the liver were focal hepatocyte necrosis and alterations of the bile ductule epithelium. Dose- and duration-dependent renal pathologies were most evident in proximal tubules, alterations which ranged from brush border damage to complete desquamation and necrosis of the epithelium. Other pathologies observed in kidney include: inflammation, hemorrhaging, alterations of distal tubules and collecting ducts, tubule dilation, foci of increased abundances of pigmented macrophages, P1 hyalinization, and glomerular lesions. As demonstrated by histopathological analysis and increases in concentrations of serum lipid peroxides, all concentrations of U in the diet resulted in significant pathologies in whitefish following prolonged exposure. These latter indices of toxicity would be useful for assessing fish health in U biomonitoring programs.

THE PHYSIOLOGY OF METAL ACCUMULATION IN RAINBOW TROUT

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This study examined acclimation to waterborne metals in juvenile rainbow trout and assessed the physiological costs of such acclimation. Trout (1-2 g) were exposed for 30-60 d to sublethal levels of Cd, Cu or Zn (150 and 450 µg/L for Zn; 20 and 60 µg/L for Cu; and 3 and 10 µg/L for Cd). Level of acclimation was determined from LT_{50} (median lethal time) and 96 h LC_{50} (median lethal concentration) challenge tests using the metal to which the fish had been exposed. Costs of acclimation were assessed by examining the following either during or after chronic metal exposure: mortality, growth, metabolic rate and swimming performance.

EFFECT OF SEAWATER ADDITION TO TOXICITY OF AN ALUMINUM SMELTER EFFLUENT USING RAINBOW TROUT (*Oncorhynchus mykiss*) IN THE EMBRYO/ALEVIN/FRY TOXICITY TEST

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This study evaluated the acute and chronic effects of an effluent on fish early life stage development. Occasional high levels of Al in the effluent was observed and small amounts of seawater (1.25, 2.5 and 5%) were added to the effluent being tested. The August draft of the Environment Canada "Toxicity Tests using Early Life Stages of Salmonid Fish (rainbow trout, coho salmon, or Atlantic salmon)" protocol was used with minimal deviance. This flowthrough 'EAF' test ran for 88 d with twice weekly shipments of effluent. For screening purposes an acute rainbow trout bioassay was conducted on each batch of effluent. The effluent was acutely toxic only four times during this period. The lethality was found to be associated with levels of Al above 2.2 mg/L which can occur after storm events. The addition of small amount of seawater did alleviate the acute lethality of the effluents with high Al levels. In the EAF test the 100% effluent treatment which used only non acutely lethal effluent the mortality was not affected but growth was impaired. The addition of seawater to the effluents with high Al levels did not completely protect the fish at the embryo and alevin stages against mortality and growth inhibition.

MODELLING THE BINDING OF INORGANIC MERCURY BY GILLS OF RAINBOW TROUT

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Rainbow trout (*Oncorhynchus mykiss*, ~1-5 g) were exposed to ~2 mM of inorganic mercury ($HgCl_2$) in synthetic soft water. To the water was added dissolved organic carbon (DOC) and cations (Ca^{2+}, Na^{2+}, or H^+). After 2 to 3 h the fish were removed from solution, the gills extracted, and the amount of Hg bound by the gills was measured. High concentrations of DOC (>40 mg $C{\times}L^{-1}$) enhanced Hg binding by trout gills. In contrast, for other metals we have examined, DOC always reduced metal binding by the gills. A likely explanation for the enhanced binding of Hg is the abiotic methylation of inorganic Hg by humic substances. A model to predict Hg binding by trout gills was constructed, then was tested by exposing fish in natural waters to which Hg was added. In these tests, toxicity of inorganic Hg to the fish best correlated with Hg accumulation by the gills at 4 h, and did not correlate with Hg accumulation by the gills at 1 or 3 d. Therefore, it is initial Hg accumulation by the gills which is most useful to model for predictive purposes.

PROTECTIVE MECHANISMS OF CALCIUM AGAINST THE PHYSIOLOGICAL EFFECTS OF CADMIUM AND COPPER ON RAINBOW TROUT

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Our goal is to better clarify the relationship between metal binding by gills of fish and the physiological mechanisms of metal toxicity to fish. Rainbow trout (*Oncorhynchus mykiss*, ~300 g) were exposed to a mixed metal solution of 0.15 mM Cd and 0.75 mM Cu plus 0.1 or 1.1 mM Ca (i.e. synthetic soft or synthetic hard water). Trout were cannulated via their dorsal aortae to allow repetitive blood sampling. Fish blood was monitored for ion concentrations and for blood gas and acid-base disturbances. Previously, our lab demonstrated that Ca prevented Cd binding to trout gills in short exposures (2 h), but Ca did not protect against Cd accumulation by trout gills in longer exposures (7 d) even though acute toxicity due to Cd was eliminated. In fact, approximately double the amount of Cd was bound by trout gills exposed to Cd and high Ca concentrations compared to the terminal gill Cd concentrations from trout exposed to Cd in low Ca conditions. In the present study, we predicted that Ca would prevent Cd and Cu accumulation in trout plasma and would prevent Cd and Cu induced respiratory gas and ionoregulatory disturbances, despite Cd accumulation on trout gills.

ACUTE TOXICITY OF ORGANICALLY-COMPLEXED COPPER TO RAINBOW TROUT

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Cu bioavailability and toxicity to early life stage rainbow trout (*Oncorhynchus mykiss*) was evaluated in laboratory toxicity tests performed using organic acid mixtures. Geochemical modelling was used to design exposure solutions which would simulate dissolved organic carbon (DOC) of a natural aquatic system, and to determine Cu speciation during bioassays. Failure time modelling indicated that mortality was associated both with inorganic Cu and with organically-complexed Cu. Specifically, Cu complexed with lower affinity ligands ($\log K_{Cu} < 6.75$) contributed to lethality, whereas Cu complexed with a high affinity ligand did not. Estimates of 96 h LC_{50} determined at widely varying levels of DOC (0-16 mg/L) were extremely consistent (8.3-9.7 µg/L) when modeled using the sum of inorganic Cu and Cu bound to low affinity ligands. Our results indicate that organically-complexed Cu can be acutely toxic; the acute toxicity of this organically complexed Cu apparently is determined by the binding affinities of specific organic ligands relative to that of the fish gill.

COPPER UPTAKE KINETICS AND CRITICAL GILL COPPER CONCENTRATIONS IN CHINOOK SALMON FRY

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One approach to evaluating metal bioavailability/toxicity to fish is to treat the gill as an organic ligand and predict metal accumulation on the gill. Gill residues then are presumed to be related to acute lethality at some critical toxicant concentration. We performed an experiment to evaluate Cu uptake and gill Cu residues in survivors versus moribund fish. We exposed chinook salmon (*Oncorhynchus tshawytscha*) fry for 48 h to 100 µg/L Cu in soft water. Ten fish were sampled at test initiation and after 1, 2, 4, 8, 24 and 48 h. In addition, we sampled aquaria every 30 min (from 8-48 h) to remove dead/moribund fish with minimal interference from post-mortality Cu accumulation. Gills were immediately excised and gill-Cu concentrations determined by GFAAS. Cu concentrations on the fish gill increased dramatically over the first 4 h of Cu exposure from 28 to 266 nmol Cu/g dry wt. Gill-Cu concentrations subsequently decreased to 100 nmol Cu/g dry wt, presumably due to activation of compensatory mechanisms. This pseudo-equilibrium between the fish gill and the water occurred after 24 h Cu exposure. The kinetics of Cu uptake in different water types was not examined. Dead fish had gill-Cu concentrations similar to live fish after 24 and 48 h Cu exposure. We discuss uptake kinetics and whether these data support the critical residue concept.

Mining Studies

THE APPLICATION OF THE REFERENCE CONDITION APPROACH TO EEM FOR THE METAL MINING INDUSTRY

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Traditional methods of establishing control sites in field-oriented biomonitoring studies of water quality are limited. The reference-condition approach offers a powerful alternative because sites serve as replicates rather than the multiple collections within sites that are the replicates in traditional designs using inferential statistics. With the reference-condition approach, an array of reference sites characterises the biological condition of a region; a test site is then compared to an appropriate subset of the reference sites, or to all the reference sites with probability weightings. In this paper we provide an overview of the development of reference data bases in two large ecosystems, the Great Lakes and Fraser River basins. The accuracy of predictive models of invertebrate community structure are shown to be more than 70% using a few easily collected environmental attributes and the application of the models for predicting metal related effects in Collingwood Harbour, Lake Huron and in a small stream in the Thompson River basin, British Columbia, are demonstrated.

DEVELOPMENT OF A SUBLETHAL BEHAVIORAL BIOASSAY FOR MINING EFFLUENTS USING THE HETEROTROPHIC FLAGELLATE, *Polytomella papillata*

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An aggregation inhibition bioassay using the heterotrophic flagellate, *Polytomella papillata*, has recently been used to assess the toxicity of several reference toxicants and pulp and paper mill effluents. The test method involves comparing the relative time that it takes for the test organisms to aggregate in an exposure test tube, in a series of toxicant concentrations. The proposed test is rapid, and does not require any microscopy for cell enumeration, a common obstacle for many micro-scale tests. An obvious extension of this bioassay is as a tool in evaluating mining effluents. We have developed this test further by: [1] examining the reproducibility of the test with five metal mining reference toxicants; [2] determining the sensitivity of the test, correlated with various toxicants in mining effluents; and [3] investigating the sensitivity of the test, compared to other sublethal tests used to evaluate mining effluents in Canada. Zinc sulphate, cadmium nitrate, lead nitrate, copper sulphate and sodium isocyanate were investigated as potential reference toxicants; and aggregation behaviour of the flagellates in response to these metal salts will be described. A full description of the test method, and the results generated during the study, performed to date, will be presented. Recommendations for

the future work with the test method, including method refinement and further standardization, will be discussed. Partial funding has been provided by the National Biotechnology Strategy Program, Natural Resources Canada.

THE USE OF THE SEDIMENT TRIAD IN EVALUATING THE BIOLOGICAL IMPACT OF ZINC MINE CONCENTRATES AT TWO ATLANTIC HARBOURS

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A 'triad' approach to sediment bioassessment is based on weight-of-evidence of sediment quality impact using sediment chemistry, sediment toxicity, (sometimes including bioaccumulation testing) and benthic community analysis. We recently used the sediment triad approach at two former Zn concentrate handling facilities in Atlantic Canada where levels of Zn and Cd exceeded Environment Canada's Interim Probable Effect Levels (PELs). The studies included: determination of sediment metal concentrations, batteries of three to five marine sediment toxicity tests, assessment of sediment bioaccumulation potential evaluations, and benthic macroinvertebrate community structure. The studies focused on the areas adjacent to the former concentrate handling facilities, where sediment concentrations of Zn and Cd were substantially higher than the PELs. Although these concentrations indicated the potential for adverse biological effects, the weight of evidence suggested that biological impact of affected sediments was minor. The absence of substantial toxicity in laboratory bioassays and lack of benthic community effects was consistent with the low porewater metal concentrations and the relatively inert, biologically-unavailable, sulphide forms of Zn and Cd associated with the Zn concentrate. In both cases, we concluded that little or no biological benefit would be realized by removal of the metal-enriched deposits.

USING BIOACCUMULATION AND GROWTH IN FRESHWATER BIVALVES AS A MONITORING TOOL FOR MINING EFFLUENTS IN CANADA

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In an evaluation of freshwater bivalves as a biomonitoring tool for assessing mining effluents in Canada, it was determined that bivalves can and should be used as indicators of exposure and effects. This approach is cost-effective, scientifically defensible, and technically feasible. The synoptic characterization of exposure from metal bioaccumulation and characterization of effects from sublethal endpoints like growth makes the use of bivalves a potentially powerful monitoring tool. Transplanting caged bivalves facilitates measuring endpoints like bioaccumulation and growth and increases the flexibility for manipulative field testing and hypothesis testing. Several literature examples were found demonstrating that significant changes in the relationship between metal concentrations in bivalve tissues and metal concentrations in water or sediment were associated with measured adverse effects. Effects for Cu and Cd could be predicted by a change in the bioconcentration factor (BCF). For Cu, the tissue burdens associated with adverse biological effects were also surprisingly similar across species and similar between freshwater and marine environments. For Cd, adverse effects were predicted from two significant changes in the relationship between Cd in sediment and Cd in bivalve tissues. Using these changes in BCF from sediment chemistry and bivalve tissue chemistry data as a criterion, the predicted sediment Cd threshold level and probable effects level were very close to interim Canadian freshwater sediment quality guidelines. Although it is clear the bioaccumulation in itself is not an effect, it is encouraging that these relationships demonstrate the possibility of predicting first-order approximations of where effects might occur from bivalve tissue burdens.

THE USE OF METALLOTHIONEIN AS A BIOMARKER IN MINING PROGRAMS FOR METAL MINING

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Metallothioneins (MT) are low molecular weight, cysteine-rich metal-binding proteins that show a high affinity for Group IB and IIB metal ions. Given their molecular properties and the present knowledge on their roles in metal uptake, regulation, and detoxification, researchers have attributed a considerable potential to these proteins as contaminant-specific biomarkers of metal exposure and/or stress. The present evaluation of MT as a biotool is based principally on published field studies performed in mining regions. Main conclusions of this evaluation are: [1] MT can already be considered to be a useful biomarker of exposure to certain metals. Indeed, strong field evidence (15 studies) supports the fact that MT responds in a dose-dependent manner to changes in ambient levels of a trace metal or of a group of trace metals (e.g. Cd, Cu, Zn, Ag); [2] use of MT as a means of evaluating metal effects on cells, organisms and populations is less well established. Only 4 field studies examined this issue - high MT levels were associated with deleterious effects at the organism and population levels of biological organization. There is a need for fundamental, mechanistic research on understanding the role of MT in metal toxicology. [3] MT is not a 'stand on its own' tool. As for any monitoring tool, MT level in an organism has to be used in conjunction with other biotic and abiotic measurements to be interpreted unambiguously.

THE ENVIRONMENTAL IMPACT OF A TAILINGS WATER SPILL: OMAI GOLD MINES

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At approximately midnight on the 19 August 1995, a breach in the tailings dam at the Omai Gold Mines Limited(OGML) in Guyana, was observed by a worker carrying waste rock to the waste rock dump. The spill was reported to management which in turn mobilized an emergency response team to rectify the problem and to mitigate the downstream impact. A sampling program was initiated within 4 h of the spill discovery which involved sampling of both the Omai and Essequibo Rivers. Samples were collected and analyzed for cyanide and heavy metals along transect lines established over the entire length of the Essequibo River from the OGML site to the where the river discharges into the Atlantic Ocean. The analytical results were then used to determine what impact the tailings water spill had on the river, both with view to environmental effects and human health issues. Once the spill was contained, a clean up program was initiated to collected deposited materials in the Omai River basin. The purpose of this clean up was to avoid the movement of contaminated materials into the Essequibo River, as well as removing deposits located in the Omai River. Approximately two mon after the spill, a study was performed to determine if the spill had any impact upon the bottom sediments and the benthic organisms that inhabit the river. The results of this study revealed no difference in the metal concentrations of the sediments in comparison to metal concentrations found in river sediments located upstream of the spill. Results of benthic invertebrate identification demonstrated a paucity of organisms throughout the Essequibo River system with no identifiable impact as a consequence of the spill. A clearly defined impact of the spill was noted in the Omai River.

CANADIAN PROGRAM ON AQUATIC EFFECTS TECHNOLOGY EVALUATION (AETE)

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The mandate of the Aquatic Effects Technology Evaluation (AETE) Program is to evaluate environmental monitoring technologies to be used by the mining industry and regulatory agencies in assessing the impacts of mine effluents on the aquatic environment. The Program includes three main technical areas: acute and chronic toxicity testing, biological monitoring in receiving waters, and water and sediment monitoring. AETE is a cooperative program between the government and the industry. The program has two main objectives: [1] to assist the Canadian mining industry meeting its environmental effects monitoring and related requirements in as cost-effective a manner as possible; and [2] to benefit the Canadian environment by evaluating new and existing monitoring technologies for the assessment of environmental impacts, indicating the benefits and limitations of each technology and documenting the estimated costs of implementation. The program activities are of two types: [1] technical evaluations based on critical review of the scientific literature; and [2] field surveys and laboratory evaluations of selected methods at five mine sites (case studies). The deliverables will be a series of published reports and a synthesis report to recommend specific methods or groups of methods that will permit accurate characterization of environmental impacts in an overall monitoring strategy. Overview on some results and recommendations obtained up to now on different techniques will be presented.

ECOSYSTEM RECOVERY IN THE ONAPING RIVER, SUDBURY, ONTARIO

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Rich mineral deposits were first discovered in the Levack area, approximately 45 km west of Sudbury in 1887. The first mine began operation in 1913, and by 1977 there were 9 active mines in the area. Mines use large quantities of water during the processing of mineral ore and extraction of minerals. In addition, surface and groundwater runoff from waste rock piles and tailings areas often accumulate high levels of metals and other contaminants. Biological impacts can occur when untreated process and runoff water enter the nearest surface receiving waters. Prior to 1954, effluent and tailings were discharged directly into the Onaping River with no treatment. This paper provides an overview of some of the historical impacts of mining on the Onaping River and subsequent changes in the ecosystem due to improved methods of waste treatment.

The Falconbridge mining operation at Levack discharges excess water into Moose Lake which overflows through Moose Creek. This small stream flows for approximately 4 km where it enters the Onaping River (Fig. 1). The Onaping River at this point also receives wastewater from other mines in the area as well as the Levack sewage treatment plant. The first biological study in the Levack area was completed in 1965 by the Ontario Water Resources Commission, the forerunner to the Ontario Ministry of Environment and Energy (MOEE). These studies reported that Moose Creek was in a 'deplorable condition' and that the quality of the Onaping River was visually impaired below the town of Levack. Work was soon undertaken to construct the Moose Lake tailings area complete with an oxidation pond and limestone neutralization pond for the precipitation of metals. The chronology of various treatment applications and introduction of environmental legislation for the mining sector are summarized in Table 1.

During the mid 1970s it was observed that pH of the Onaping River was severely depressed downstream of the mine discharges (Bolger, 1980). The pH effect had gone undetected since

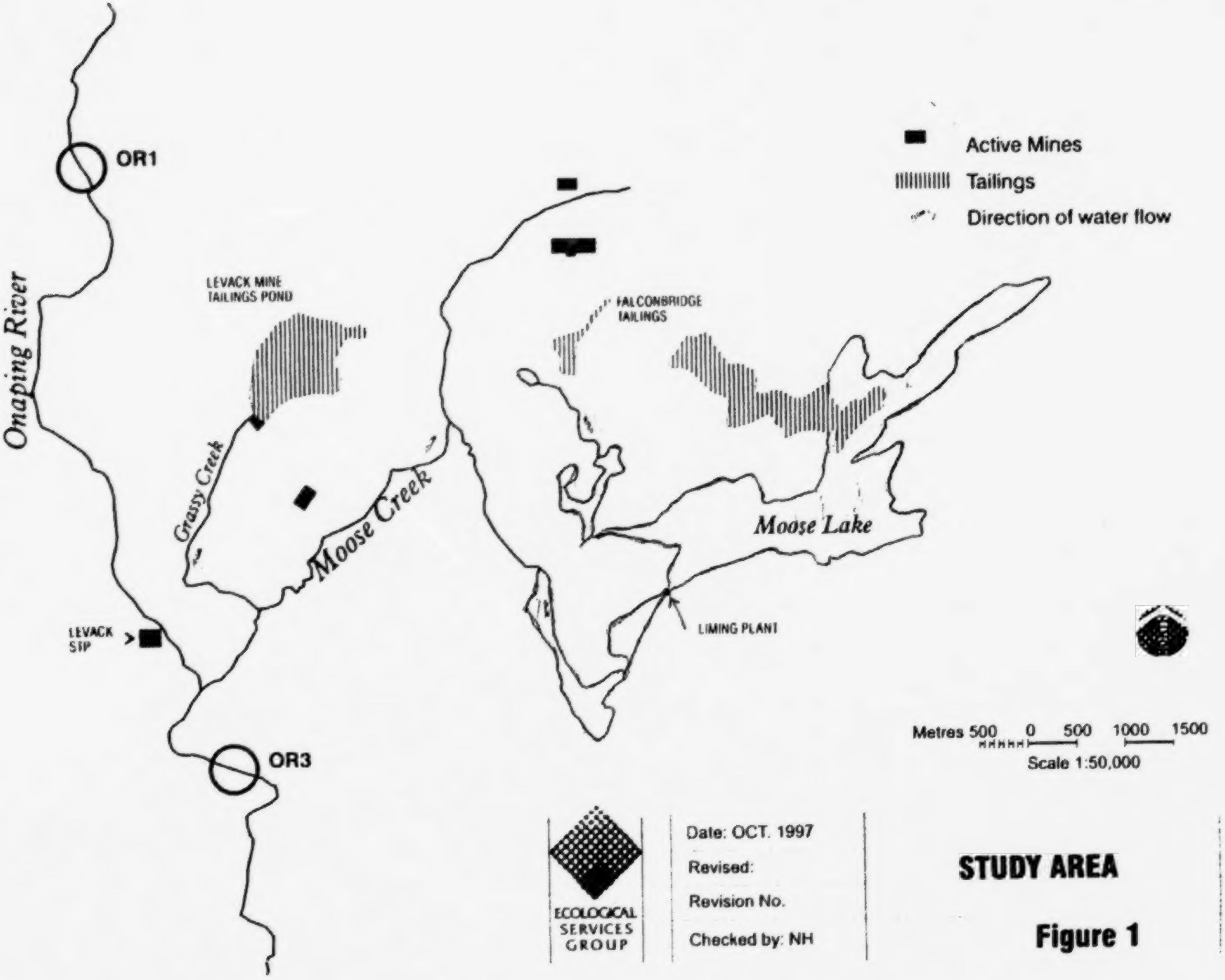
Onaping River
OR1
OR3
Active Mines
Tailings
Direction of water flow
LEVACK MINE TAILINGS POND
FALCONBRIDGE TAILINGS
Grassy Creek
Moose Creek
Moose Lake
LEVACK STP
LIMING PLANT
Metres 500 0 500 1000 1500
Scale 1:50,000
ECOLOGICAL SERVICES GROUP
Date: OCT. 1997
Revised:
Revision No.
Checked by: NH
STUDY AREA
Figure 1

Fig. 1. Study area showing downstream sample location (OR3) in Onaping River.

Table 1. Chronology of events and mine waste treatment in the Levack area.

Period	Event/Treatment Method
Prior to 1954	No treatment. Waste discharged directly to Onaping River.
ca. 1954	First concentrator plant. Tailings ponds used for removal of suspended solids.
1968	Construction of Strathcona Mill and Moose Lake tailings area with limestone neutralization plant. Moose Lake separated into 3 treatment basins: (a) tailings pond; (b) oxidation pond; (c) neutralization/metal precipitation pond.
1977	Metal Mining Liquid Effluent Regulations (MMLERs)
1977	Severe pH depression observed in Onaping River due to partially oxidized sulphur compounds. In response to this, effluent from other mines directed through Moose Lake for greater retention time.
1982	Construction of dam at Fecunis Lake to intercept drainage from Strathcona drainage.
1989/90	MISA program for Ontario Mining Sector.
1990	New pumphouse at Fecunis Lake dam to improve interception of contaminated runoff: - upgrade of neutralization plant to use quicklime; - installation of CO_2 system to reduce effluent pH

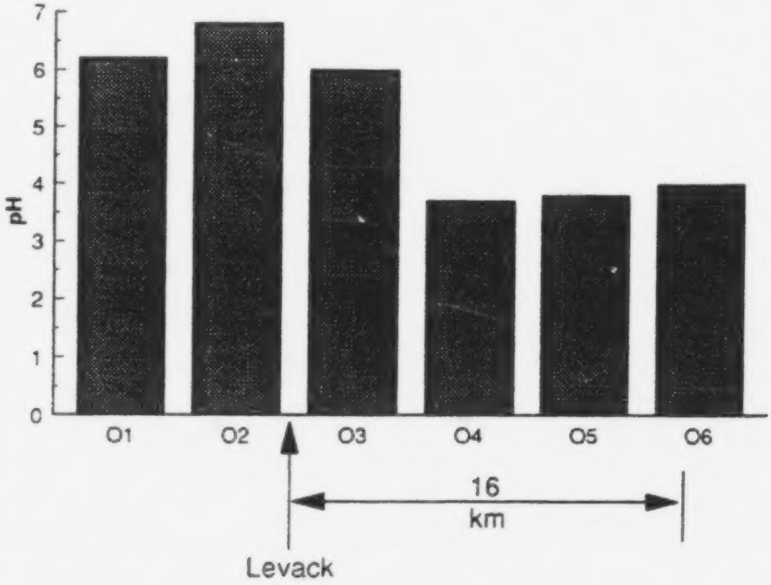

Fig. 2. Onaping River pH depression in July, 1977, at Levack.

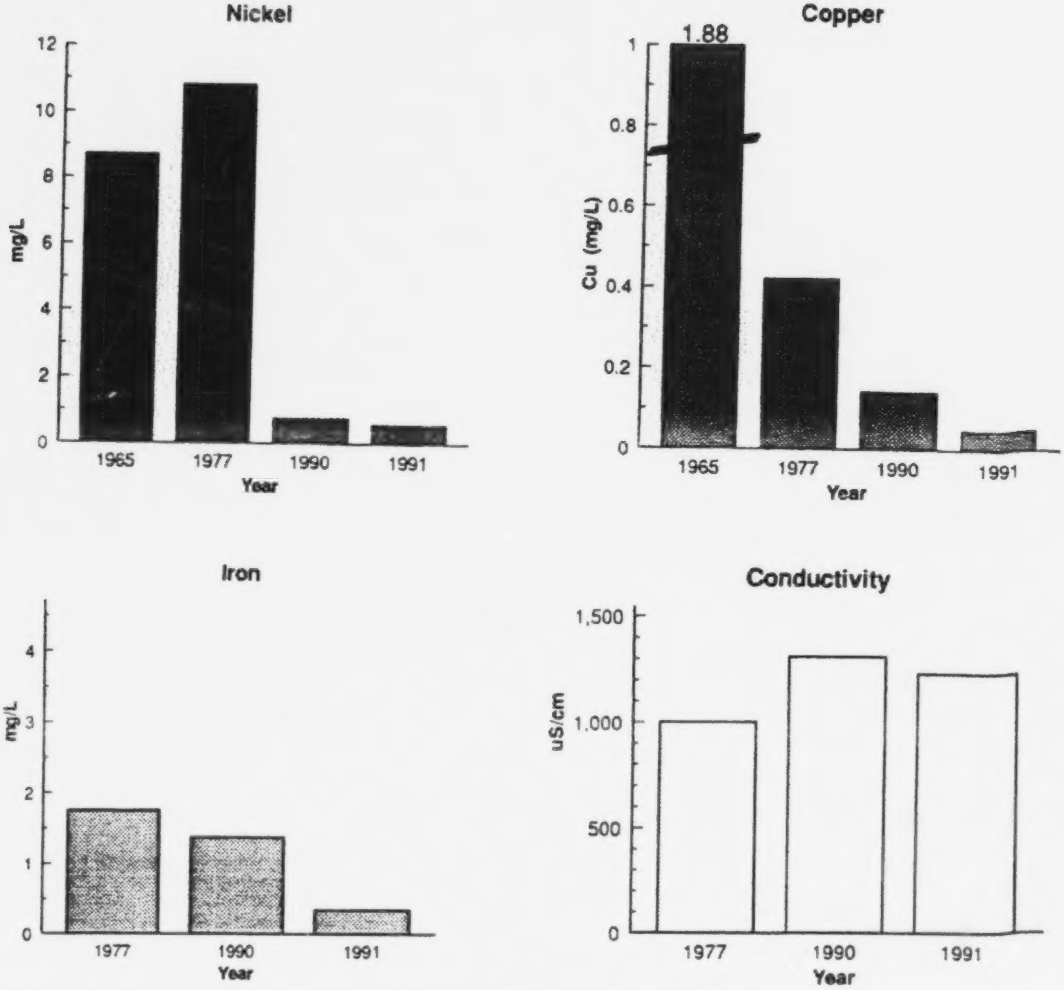

Fig. 3. Moose River water quality, 1965 to 1991, station M2.

wastewater from the Moose Lake facility was about neutral pH. However, several km downstream of the mine discharges the pH was as low as 3.7 on occasion. The low pH effect persisted for tens of km downstream (Fig. 2). It was subsequently determined the effect was due to partially oxidized sulphur compounds in the tailings water. When released to the environment, these sulphur compounds underwent further oxidation which lowered both the oxygen content and pH level of the river. Since the oxidation process took several hours to occur, the effects on the river were not apparent for several km.

The pH problem was immediately rectified by increasing the water retention time in the tailings areas and treating with oxygen. Other mines in the vicinity also routed tailings wastewater through the Moose Lake facility for added treatment. Biological effects on the river up to this time could have been due to a combination of low pH, low oxygen and elevated metal levels.

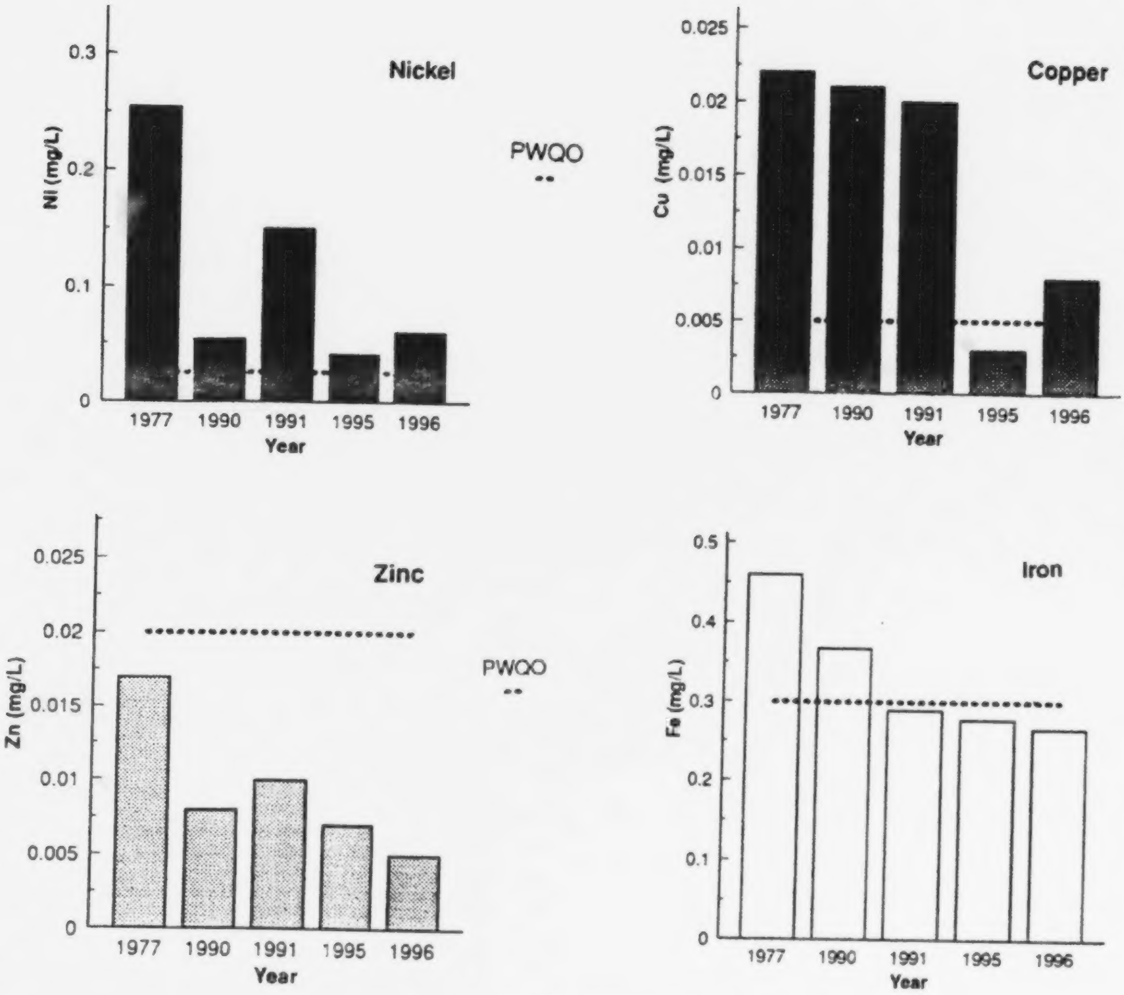

Fig. 4. Downstream water quality Onaping River, 1997 to 1996, station OR3.

The concentration of metals in Moose Creek began to decline with the implementation of different wastewater treatment. In addition, measures were taken to better control surface runoff from the area. In 1965, the average Ni concentration in Moose creek was almost 9.0 mg/L, while the average Cu concentration was 1.88 mg/L. By 1990 the concentrations of these metals declined to below 1.0 mg/L and 0.1 mg/L, respectively (Fig. 3). Total metal loading also declined proportionately. During the 1980's Ni loading from Moose Lake ranged from 10,000 to 40,000 kg/yr. Total Ni loading was down to an estimated 830 kg/yr in 1995.

Metal concentrations also showed significant reduction in the Onaping River during this time period. At the first downstream station (OR3) the concentration of Ni declined from an average of 0.25 mg/L (n = 10, range: 0.016 - 0.84 mg/L) in 1977 to an average of 0.041 (n = 23, range: 0.011 - 0.087 mg/L) in 1995 (Fig. 3). Similarly, the average concentration of Cu declined from 0.022 mg/L (n = 10, range:

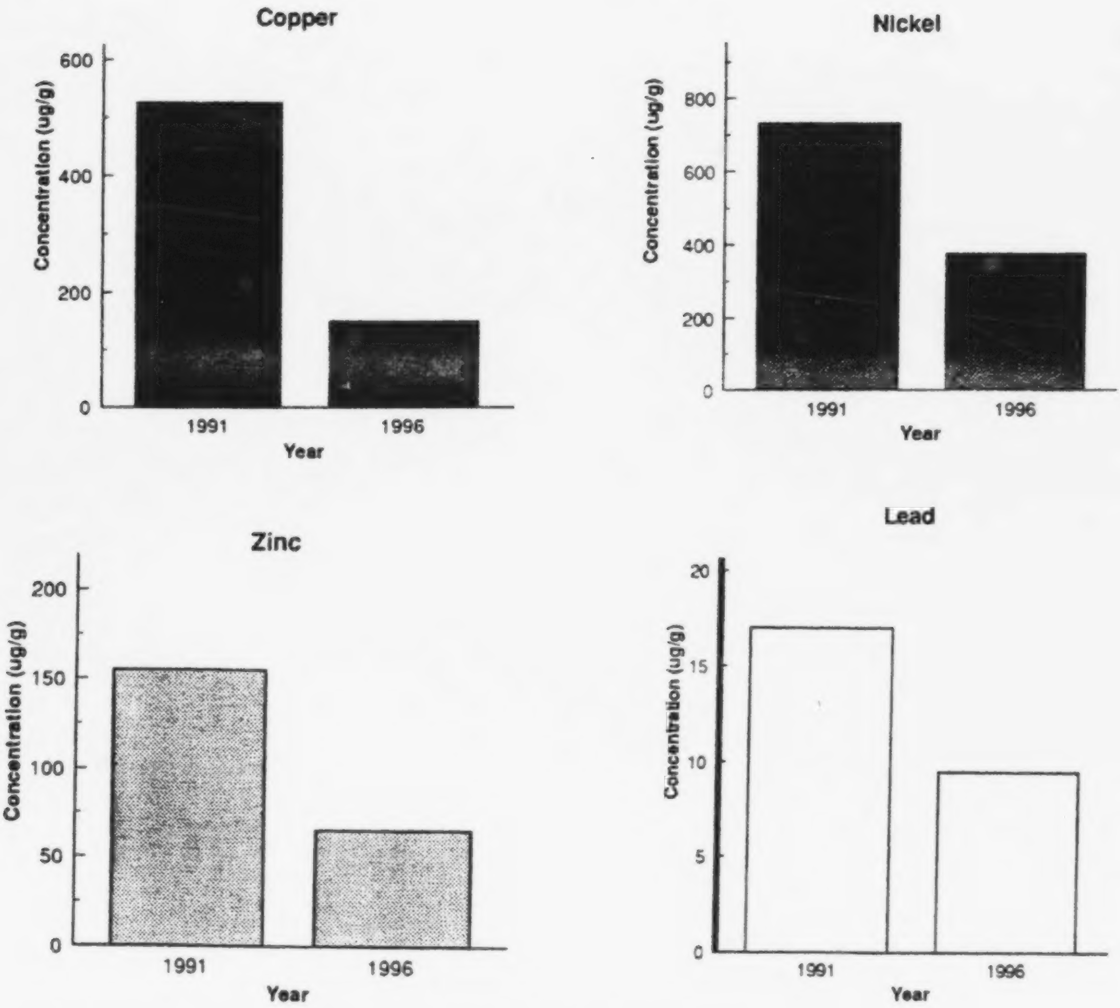

Fig. 5. Onaping River sediment quality, 1991 to 1996, station OR3.

0.003 - 0.10 mg/L) in 1977 to 0.003 mg/L (n = 23, range: < 0.0012 - 0.011 mg/L) in 1995. The level of Ni and Cu are approaching the Provincial Water Quality Objectives (PWQOs) while the levels of Zn and Fe are below their respective PWQOs as indicated by the dotted line in Fig. 4. The relative reductions in metal levels in the Onaping River is not as great as in Moose Creek. This is due to other continuing inputs of metal into the Onaping River from point and nonpoint sources.

There are limited sediment quality data for the Onaping River. This may be a reflection of the erosional nature of the river such that depositional sediments are limited and difficult to collect. However, sediment samples collected at the downstream station OR3 do reflect reduced metal loading to the river. Between 1991 and 1996 the average Cu and Ni sediment levels declined from 520 and 725 mg/kg in 1991 to 150 and 375 mg/kg, respectively (Fig. 5).

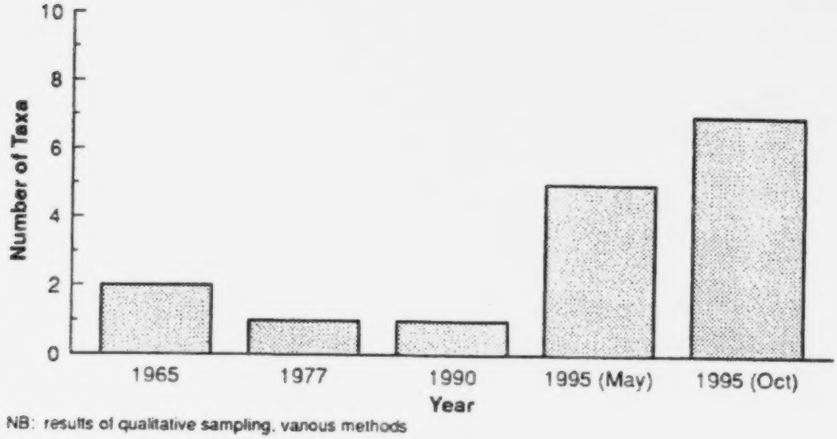

Fig. 6. Number of benthic taxa in Moose Creek 1965 to 1995, station M3.

Benthic invertebrate surveys were conducted in Moose Creek and the Onaping River in 1965, 1977, 1985, 1990, 1991, 1995 and 1996. A variety of techniques were used for collection including Surber samplers, Ekman grabs, airlifts and artificial substrates. For the purpose of this analysis, only samples collected by Surbur in the fall were compared. In addition, the taxonomic information was grouped to a similar level of identification to account .for differences in the taxonomic levels of invertebrate identification.

A survey of Moose Creek in 1965 found only two benthic taxa, consisting of chironomids and tubificids (Fig. 6). Subsequent surveys in 1977 and 1990 only reported chironomids. However, the number of taxa increased to 5-7 (spring/fall surveys) by 1995. These included individuals of Trichoptera and Odonata, benthic groups generally considered to be sensitive to degraded habitat conditions. While a total of 7 invertebrate taxa does not represent a highly diverse benthic community, the data indicate an increasing trend toward improving habitat conditions.

Benthic samples were also collected on several occasions at a station in the Onaping River (OR3) immediately downstream of the confluence with Moose Creek which carries the mine discharge. These surveys show a that the number of comparable taxa increased from 8 in 1965 to over 40 in 1996 (Fig. 7). There appears to be two distinct steps for increased number of taxa; in the early 1980s and after 1991. These periods coincide with periods of improvements in effluent treatment and wastewater handling. Another measurement of benthic community health is the EPT Richness Index. The 'EPT' index refers to the relative proportion of pollution sensitive stoneflies, mayflies and caddisflies. The increased EPT index in the Onaping River indicates a greater proportion of invertebrates that are not able to tolerate degraded habitat conditions.

Aquatic toxicity bioassays have also been conducted on the wastewater. The water in Moose Creek was acutely toxic to fathead minnows in 1965. It was not until 1989 that routine effluent testing began. By that time the discharge water from the tailings area was not acutely toxic to either rainbow trout or *Daphnia magna*. Sublethal 7 d tests conducted in 1996 showed the effluent had no effect on

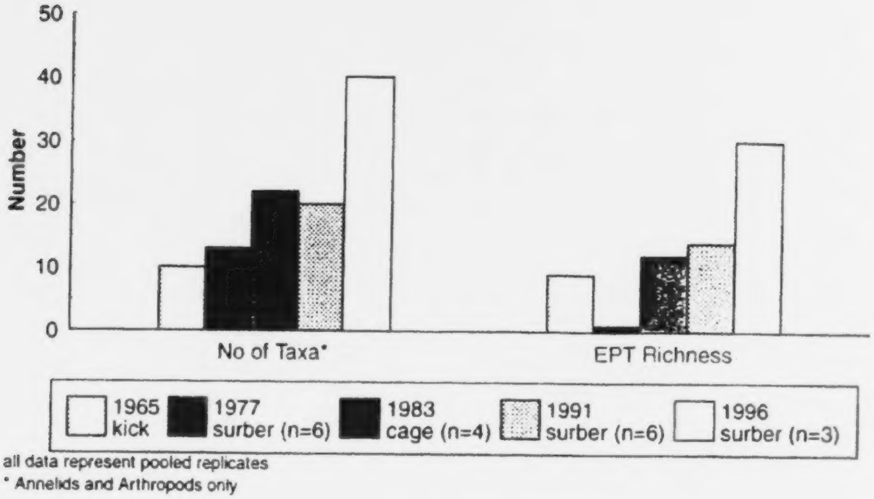

Fig. 7. Number of benthic taxa and EPT index in Onaping River, 1965 to 1996, station OR3.

growth or survival of fathead minnows. In 7 d *Ceriodaphnia* tests there was no effect on growth but there was a small effect on reproduction. The effluent also produced some effect on growth tests with *Selenastrum* and *Lemna minor*. The bioassay results show that significant improvements have been made in eliminating acute toxicity and producing an effluent that displays little sublethal toxicity.

Fisheries surveys have been conducted on the Onaping River in 1977, 1984, 1991, 1995 and 1996. These surveys have utilized different types of gear during different seasons so it is difficult to make quantitative comparison of the results. In general, relatively few fish have been caught either downstream in the exposure area or in the upstream reference area. The upstream reference area still supports a naturally reproducing brook trout population. The early studies generally caught only pollution tolerant species downstream of Moose Creek including white suckers, brook sticklebacks and brown bullhead catfish. However, during the past two surveys some specimens of mottled sculpin and rainbow trout were captured. These species are considered more intolerant of degraded conditions and again support the trend toward improving habitat quality in the downstream reaches of the Onaping River.

SUMMARY

The Onaping River has received mining effluent for over 80 yrs. Effluent was untreated for the first 40 yrs which resulted in poor water quality including low pH, low oxygen and high metal levels. Identification of these impacts lead to increased effluent treatment and improved handling of surface runoff. Metal levels have steadily declined in the receiving waters. During the past decade the benthic invertebrate community has demonstrated increased community diversity and increased numbers of pollution intolerant species. Sensitive fish species have also started to reappear. The information stresses the importance of consistent biological monitoring programs to document ecosystem responses to effluent treatment.

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DEVELOPING BIOCRITERIA FOR COMPLIANCE AND EARLY-WARNING INDICATORS

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Most effects monitoring programs utilize statistically significant differences in mean responses between reference locations and presumed impacted locations. This approach can be criticized because the detection of effects, and consequently the interpretation that a site is degraded and in need of rehabilitation, is dependent on the number of sampled collected in the reference and impacted areas. Objective decision criteria and appropriate statistical tests are required to evaluate the relevance of effects due to anthropogenic activities. First, we define ecosystem endpoints that we wish to protect directly as 'compliance indicators.' For such compliance indicators (e.g., fish community), we propose that impacts are of little interest unless they exceed the normal range of variation in reference areas. The normal range is typically defined as the region enclosing 95% of the observations or the mean ± 2 standard deviations. In contrast, monitoring tools that are only of interest because they can be used to predict impacts on our compliance indicators are defined as 'early warning indicators. For such early warning endpoints (e.g., benthos, toxicity tests), we propose that effects are unacceptable only if they coincide with unacceptable impacts on the compliance indicator. Using fish and benthos data from small streams in southern Ontario, we demonstrate a technique for developing application-specific biocriteria for an early-warning indicator (benthos) that can be used to predict impacts on a compliance indicator (fish community composition).

BIOMONITORING WITH BENTHIC INVERTEBRATES AT MINES: SOME ALTERNATIVE SAMPLING IDEAS

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I recently had an opportunity to review field and laboratory methods for benthic invertebrate monitoring of fresh waters in the context of the Canadian mining industry. Optimal methods are those that maximize cost-effectiveness, statistical power, and especially, sensitivity. Sensitivity is important for early detection of disturbance so that corrective action can be taken before severe impairment has occurred. These three criteria can be best satisfied by a comparative sampling regime that assesses the difference among sites rather than absolute population densities. Cost-effectiveness would be greatly improved by reducing the size of individual samples and sharply increasing replication at each site because smaller samples provide better population estimates (lower variance) for common species with the same total effort. Measuring key habitat variables (depth, water velocity, substratum particle size, periphyton and organic carbon content) uniformly at each sampling point allows background variability to be partitioned out in the analysis. Simplified sampling regimes, using only a few sites and possibly incorporating sequential comparison plans, should be considered at mine sites where impairment is either unlikely or obviously severe. Sequential comparison plans can reduce costs of a biomonitoring study by >50% without sacrificing statistical rigour, but they classify the site based on a single variable. Special problems in biomonitoring in the mining industry include how to sample headwater streams (where upstream controls are not possible) and how to separate the effects of multiple stresses on the receiving water body.

THE EFFECTS OF MINE TAILINGS ON SURVIVAL AND REPRODUCTION OF POLYCHAETOUS ANNELIDS

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INTRODUCTION

The primary purpose of this study was to determine possible toxic effects of wastes from a Pb and Zn mine located near Strathcona Sound, Baffin Island, Canada, on marine organisms. It had been proposed by the company to dispose of the wastes into the marine waters of the Sound. Toxicity testing was recommended by Environment Canada as a means of determining whether or not the waste tailings would have an adverse affect on the marine environment. Polychaetes were chosen as test organisms because they are an important element in the subtidal benthic communities. Polychaetes usually constitute 30 to 60 percent of the number of species and specimens of macroinvertebrates in the subtidal marine benthos. Because of their numerical abundance and the nature of the feeding and activity modes, polychaetes are effective in moving, or turning, sediments vertically much the same as earthworms do on land. Polychaetes also constitute an important food source for many demersal fish. It was apparent, therefore, that any environmental change, such as the disposal of mine tailings, that could significantly decrease the polychaete populations could lead to a stagnation of subtidal sediments and reduce the productivity of the area.

In the operations at the Nanisivik Mine, Pb and Zn are removed from pulverized ore leaving the process waste, or tailings, in the form of a slurry. Since the Strathcona Sound was located nearby, it was considered as a possible discharge site for these wastes. It was anticipated that tidal circulation would dilute and dissipate the tailings and, therefore, cause little or no adverse effects on the marine environment. In the absence of previous data on the effect of mine tailings on polychaetes, a study was requested to determine the toxicity of mine tailings and their principal metallic components, Cd, Pb and Zn, on four representative species of this animal group. This research was conducted over 20 years ago, and since that time the effect of mine tailings on polychaetes has not been investigated, other than the final report and thesis by Gerlinger (1979), as indicated by the review by Reish and Gerlinger (1997). At the time of this study only 12 studies had been published on the acute toxic effects on polychaetes and only three on the effects on reproduction. Since that time, there has been a significant increase in the number of publications on the toxicity of contaminants on polychaetes

(Reish and Gerlinger, 1997).

The objectives of this laboratory study on the possible effects of mine tailings on polychaetous annelids were: [1] to determine the acute toxicity of Cd, Pb and Zn on four species of polychaetes at various temperatures; [2] to determine the acute toxicity of mine tailings on four species of polychaetes at various temperatures; and [3] to determine the effect of mine tailings on reproduction of polychaetes as measured by the number of fertilized eggs or young produced by four species of polychaetes at various temperatures.

MATERIALS AND METHODS

The selection of test organisms for the bioassay experiments necessitated a compromise between species which occur naturally in Strathcona Sound and those which could be used as test organisms in the laboratory. Since it was impractical to transport live animals from northern Canada and attempt to culture them in California, a selection of test organisms was made from the 10 species currently under culture by us at that time. An additional criterium was that the species must be able to reproduce under laboratory conditions within a reasonable period of time.

Species selected were: *Neanthes arenaceodentata, Capitella capitata, Ophryotrocha diadema* and *Ctenodrilus serratus.* Culture and testing procedures with these four species had been established at the time of this study and have since been published (Reish, 1980; American Public Health Association, 1995; American Society of Testing and Materials, 1996). The fertilized eggs of *N. arenaceodentata, C. capitata* and *O. diadema* are laid in mucoid tubes where they are incubated by a parent until they emerge a juveniles. Each segment of the minute species *C. serratus* dehisces and develops into a new worm. Worms were fed either the green alga *Entermorpha* sp. or Tetramin® powder.

Temperature selection was also a compromise since none of these species could tolerate the temperatures present at Strathcona Sound (~0° C). *Capitella* and *Ctenodrilus* could live at 10° C but not reproduce. All species survived and reproduced at 15° C but it required a greater period of time than at 20° C. Experiments were conducted in control temperatures of 10, 15 and 20° C.

All tests were conducted at 32.0 PPT, the salinity characteristics of the deeper waters at Strathcoma Sound.

Test Procedures

The 96 h LC_{50} was determined for the four species in sea water solutions of $CdCl_2$. $PbCl_2$ and $ZnCl_2$. Five different solutions plus a sea water control were used with each metal with the concentrations ranging from 0.15 to 18.0 (Cd), 0.05 to 5.6 (Zn) and 0.02 to 22 (Pb) mg/L. The test procedures with metals are detailed in Reish, 1980.

A single specimen of *Neanthes* and *Capitella* was placed in a plastic petri dish with 20 dishes per concentration and five test concentrations plus a sea water control. Specimens were examined at 96 h and the number of survivors noted. The ratio of SiO_2 to mine tailings for the five test concentrations was determined by preliminary tests for all four species. The ratios varied depending upon the preliminary results with each species.

For the *Neanthes* reproductive tests, four young worms were placed in a 3.78 L jar with 15 jars per test concentration and five test concentrations plus control. Each jar was filled with 2,500 mL of sea water plus a mixture of SiO_2 and mine tailings. Animals were fed approximately 0.8 g of *Enteromorpha* every two weeks; however since specimens in the higher concentrations of mine tailings either ate less food or none at all, a reduced amount of food was given or none at all. At the end of four weeks, females containing developing ova were isolated and paired with a male as determined by the absence of fighting behavior (Reish and Alosi, 1968). A total of 15 pairs were used for each concentration with one pair per test container. Each pair was examined weekly for the presence of embryos in the male's mucoid tube. The embryos were removed and counted.

The reproductive test procedures for *Capitella* were similar to that which was used with *Neanthes* with the exception that 25 worms were placed in a jar with a total of four jars per concentration. Worms were fed Tetramin® powder. Specimens within a jar were examined fr of embryos counted. Each jar was examined weekly until all females incubating embryos had been removed or had died. Males are identified by the present of genital hooks on segments 8 and 9 and females by the presence of developing ova in the coelom (Reish, 1980).

Smaller petri dishes were used for *Ctenodrilus* and *Ophryotrocha* because of their minute size. Four animals were placed in each dish with 10 dishes per test concentration. Since both of these species have short life cycles, the experiment was extended beyond the 96 h period after recording the number of survivors to continue as the reproductive test. Tetramin® powder was used as food for both species. The number of specimens present within each dish was counted at the end of 28 d for experiments conducted at 20° C and a longer period of time for experiments conducted at the lower temperatures.

Tailings from the mine was sent to a commercial laboratory in Vancouver where it was pulverized and treated in much the same manner as it would have been at the mine. The material was then shipped to California in 18.9 L plastic carboys. The carboys contained approximately 17.0 L of fresh water supernatant and 1.9 L of solids. The supernatant was decanted, and the solids oven dried at 50° C. The dried solids were thoroughly mixed, sea water added and allowed to settle for 48 h at which time the sea water was drawn off for chemical analysis. The dried mine tailings and the fresh water and the sea water supernatants were analyzed for six metals as specified by Environment Canada (Table 1). The concentrations of the six metals in the mine tailings were high, and with the exception of As. They were higher in the sea water supernatant compared to that in fresh water.

Table 1. Chemical analysis of the heavy metals present in the Strathcona Sound mine tailings, fresh water and sea water supernatants.

Element	Mine Tailings (mg/kg)	Fresh Water Supernatant (mg/kg)	Sea Water Supernatant (mg/kg)
Arsenic	180.0	3.2	0.016
Cadmium	28.0	<0.02	0.269
Copper	370.0	0.22	46.0
Iron	220,000	0.43	11.0
Lead	2,600	1.1	24.0
Zinc	11,000	0.2	420.0

Preliminary experiments were conducted to determine if the mine tailings were toxic and, if so, at what concentration. Initial tests indicated that the mine tailings were extremely toxic and that it would be impossible to conduct any toxicity tests with mine tailings and sea water only. It was necessary to add SiO_2 powder (<0.044 mm diameter) as an inert compound in varying ratios with the mine tailings. It was determined that SiO_2 was not toxic; however, a SiO_2 control in addition to a sea water control was used in these tests. Test ratios of SiO_2 to mine tailings for the 96 h tests varied according to preliminary tests: 200:1 to 2000:1 (*Neanthes*). 5:1 to 2000:1 (*Capitella*), 10:1 to 2000:1 (*Ophryotrocha*) and 200:1 to 2000:1 (*Ctenodrilus*). The amount of mine tailings was less for the reproductive test with *Neanthes.*

RESULTS AND DISCUSSION

The biological effects are summarized in Tables 2 and 3. Table 2 records the 96 h LC_{50} of sea water solutions in mg/L of Cd, Pb and Zn to the four species polychaetes. Table 3 gives the 96 h LC_{50} of a SiO_2 mine tailing mixture (as ratios) and the concentration at which reproduction is suppressed for the four species of polychaetes by test temperature.

Zn was the most toxic to all species at both temperatures with the possible exception of *Capitella*. Cd was the least toxic at both temperatures. Overall, *Ophryotrocha* was the most sensitive species and *Neanthes* was the least sensitive of the four species to these three metals (Table 2).

The effect of mine tailings on survival and on suppression of reproductions are summarized in Table 3 by species and by temperature. *Neanthes* was the most sensitive of the four species at both temperatures, and *Capitella* was the least sensitive species. *Capitella* and *Ctenodrilus* were the only species able to live at 10° C, but they did not reproduce. A relationship between toxicity and water temperature was noted; worms were more sensitive at the colder temperatures which is probably the result of the stock colony being cultured at 20° C.

The concentrations of the sea water supernatant (Table 1) of cadmium is less than the 96 h LC_{50} to all four species; the concentration of Pb is slightly higher than the 96 h LC_{50}, but Zn is over two orders of magnitude higher than the 96 h LC_{50}s to all species. The syngeristic effect of all metals plus other constituents not measured undoubtedly played a role. For example, the 96 h LC_{50} to these four species ranged from 0.16 to 0.3 mg/L, which compares to the concentration of 46 mg/kg in the sea water supernatant (Reish, 1980; Table 1).

The effects of the SiO_2-mine tailings mixtures at two test temperatures are summarized in Table 3. The LC_{50} data are given as the ratio of SiO_2 to mine tailings with the amount of the SiO_2 being constant in all experimental containers within an experiment. Therefore, as the amount of mine tailings is increased, the ratio of SiO_2 to mine tailings is decreased. *Neanthes* was the most sensitive species to reproduction at 15° C and similar with *Ophryotrocha* at 20° C. *Capitella* and *Ctenodrilus* were able to survive at 10° C but failed to reproduce. Reproduction was less at 15° C than at 20° C in both the number of animals which reproduced and in the number eggs laid (or segments in *Ctenodrilus*). This was similar to what Reish and Gerlinger (1984) noted with *Neanthes* in studies with the same metals.

Table 2. The 96 h LC_{50} of Cd, Pb and Zn to four species of polychaetes (in mg/L) at two temperatures.

Species	Cadmium		Lead		Zinc	
	15°C	20°C	15°C	20°C	15°C	20°C
Neanthes	17.8	12.1	10.7	7.7	1.4	1.8
Capitella	5.1	11.7	<2.9	<7.3	8.0	10.7
Ophryotrocha	11.9	1.6	4.8	1.7	3.5	1.2
Ctenodrilus	4.1	3.7	12.8	*	1.8	1.8

*No deaths at 2.95 mg/L, the highest concentration tested.

Table 3. The 96 h LC_{50} of silicon dioxide-mine tailing mixture and the concentration at which reproduction is significantly suppressed to four species of polychaetes.

Species/Temperature	96 h LC_{50}	Suppression Concentration
Neanthes[1]		
15° C	502:1	4000:1
20°	435:1	1500:1
Capitella		
10°	<200:1	---[2]
15°	13:1	<1000:1
20°	15:1	<1000:1
Ophryotrocha[1]		
15°	74:1	500:1
20°	30:1	1500:1
Ctenodrilus		
10°	700:1	---[2]
15°	235:1	1750:1
20°	380:1	1000:1

[1]All worms died at 10° C

[2]No reproduction at 10° C

The effect of mine tailings on toxicity and reproduction on polychaetes had not been studied previously to this study nor since. The relationship between the 96 h LC_{50} and reproduction and a metal varies between similar results to over two orders of magnitude depending on the metal. Since the mine tailings were found to be very toxic, it was necessary to mix an inert compound (SiO_2) with the waste in order to measure an effect. The 96 h LC_{50} and the concentration to cause suppression of reproduction indicated that these mine tailings were very toxic and would be detrimental to the benthic fauna if discharged into Strathcona Sound. The decision not to discharge the wastes into the Sound demonstrated the usefulness and importance of conducting these toxicity tests. It was suggested that a nearby Arctic fresh water pond be drained and the mine tailings be discharged into the empty pond; the permafrost would prevent movement of the elements into the surrounding environment. It is our understanding that this was the decision made by Environment Canada.

This research was supported by a contract from Environment Canada, Winnipeg.

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METAL MINING EEM: THOUGHTS ON DECISION MAKING

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Based on the recommendations of the Assessment of the Aquatic Effects of Mining in Canada (AQUAMIN), Environment Canada is leading the implementation of an Environmental Effects Monitoring (EEM) program for metal mining. Absolute certainty is not possible in EEM, so in making decisions, environmental managers must weigh risks and consider uncertainty. To assist environmental managers, it is recommended that a decision making framework be established for metal mining EEM. Such a framework would make the decision making process more systematic and rigorous, as well as more inclusive, transparent, and accountable. The framework would help ensure effective study design, an important step to obtaining high quality data. The framework would also include tools for data interpretation, including the weight-of-evidence approach, and tools for quantitative consideration of uncertainty, such as decision analysis. Considering the EEM data, as well as policy and socio-economic factors, decision makers can apply these tools to choose the best management option in response to EEM results.

SPECIATION OF METALS IN ENVIRONMENTAL MEDIA: AN IMPORTANT MODIFIER OF TOXICITY

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The uptake of Cd, with specific reference to speciation of this metal in the micro environment of the interface (gill, root or gut) between organism and environmental medium, has been the focus of a Canadian Network of Toxicology Centres research team. Methods to measure 'available' Cd (Cd $^{2+}$) in diverse aquatic media containing dissolved organic matter have been developed and integrated into work with vegetation and human cells, as a critical part of describing biological studies in terms of the amount of Cd to which organisms are being exposed. Uptake and accumulation of Cd by vegetation is being studied relative to its speciation in the root environment and modification of the rhizosphere by root activity, in order to better predict the uptake of Cd by plants, and the subsequent transfer of Cd to humans through diet. Kinetics of Cd uptake by human intestinal epithelial cells are being studied relative to other ligands commonly found in gut content. Kinetics and accumulation of Cd in fish gills and alga are being studied relative to dissolved organic matter and complexing ligands in aquatic media. In all three biological systems, the focus is on how speciation or complexation of Cd in the microenvironment relates to the observed effect, and how the biological system may modify the microenvironment, resulting in more or less bioavailable Cd relative to the macroenvironment. These data will lead to better predictions of Cd toxicity to the environment by relating effect of bioavailable metal rather than total metal, thus contributing to the development of more rational environmental quality standards of Cd. Cd was chosen as the metal of focus for its ubiquity in both occupational and agricultural environments, its suspected links to human health effects and its inclusion in CEPA's Priority Substances List 1.

METALS IN THE ENVIRONMENT (MITE): A RESEARCH PROGRAM

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The future use of metals in the world's economy is currently the subject of some considerable debate, particularly within the European Economic Community. The Canadian Network of Toxicology Centres (CNTC) in collaboration with the Mining Association of Canada organized a workshop in October 1996 to consider the research needed to estimate the long term risk to ecosystems from metals associated with mining, metallurgy and manufacturing. The discussions focused on three themes: [1] natural and anthropogenic sources of metals; [2] transport and transformation of metals; and [3] fate and impacts of metals. These three themes were seen to be inextricably linked. For example, metals introduced into the earth's surface environment, whether as the result of natural or anthropogenic processes, will be subjected to various transformation and transport processes. For any give metal, the nature and dynamics of these processes will be affected by the metal's speciation, reciprocally, however, these processes my well alter a metal's speciation and thus affect its fate and its impact on the biotic components of the ecosystem. For each of these three themes, the general question "What is unknown?/What must be known?" was broken down into smaller, more specific needs for information. The overall identified need was to determine the relative contributions of natural and anthropogenic sources to metal loadings to the various environmental "spheres" (atmosphere, geosphere, hydrosphere and biosphere) over time and space, and to evaluate the ecological impacts of these metal loadings as well as their contribution to the non-occupational exposure of Canadians to metals. The proposed research will greatly advance our understanding of risk to human and environmental health from metals in the environment, by quantifying the anthropogenic inputs relative to inputs from geo-cycling, thus more specifically identifying the potential benefits of emission control. The proposed research will address the issue of metal-induced effects relative to the forms of metals that actually occur in the environment, rather than to the simple metal salts so often used in toxicity testing, thus more closely estimating the true environmental response to metals. The proposed research will include biological community endpoints that assess chronic exposure to metals, thus addressing the risk of sustained metal extraction and use on a global scale. The users of this information will be producers of metals and environmental regulators deciding on appropriate policies regarding the use of metals; these parties have already participated in identifying the research priorities and are in the process of committing future resources to direct or collaborative support.

EEM Pulp Mills

ENVIRONMENTAL EFFECTS MONITORING OF PULP AND PAPER MILLS: RESULTS OF STUDIES CONDUCTED AT 47 MILLS IN QUÉBEC

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As part of the first cycle of the Environmental Effects Monitoring (EEM) Program conducted under the Canadian Pulp and Paper Effluent Regulation, 47 studies were made in 1994 and 1995 by Québec mills, prior to or shortly after implementation of secondary treatment. The methods and the protocols used by the mills were generally in accordance with those prescribed by Environment Canada and Fisheries and Oceans Canada. After implementation of secondary treatment, a significant decrease of both lethal and sublethal toxicity of the mills effluent was observed and was influenced by the type of treatment. The sublethal test with the waterflea (*Ceriodaphnia dubia*) proved to be the most sensitive indicator of effluent toxicity in the inland waters, where 90% of the mills are located. When compared to an upstream reference zone, differences indicative of a degradation of the benthic community in the study zone were observed in 57% of the studies. However, confounding factors other than the mills effluent may contribute to these differences. Regarding the adult fish survey, only a few mills were able to collect the prescribed minimum sample size for two species. Nevertheless, available results showed differences between the study and reference zone at several sites, especially for female fecundity and liver weight of white sucker (*Catostomus commersonii*) populations. Where concentrations in the effluent were sufficiently high, resinic acids in fish bile were suitable as exposure chemical tracers for 56% of the mills. The dioxins and furans concentrations measured in sportfish muscles showed no difference between the reference and the study zone of the 8 mills sampled and the levels were much lower than the Canadian guidelines for fish consumption.

SUGGESTIONS FOR IMPROVING DATA QUALITY OF THE ADULT FISH SURVEY COMPONENT OF CYCLE 2 EEM STUDIES AT PULP AND PAPER MILLS

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Results obtained in the adult fish survey component of Cycle 1 EEM studies have been difficult to interpret for numerous reasons including poor catch success of sentinel species, confounding factors in receiving water bodies, and high variability of data. In addition, it has been suggested that for some studies the quality of fisheries data may be poor due to inadequate study design, inexperience of field crews, budget constraints, rushed studies due to consultants being overloaded, and lack of training in conducting complex statistical analyses. The following presentation proposes systems for mills to use for consultant selection, auditing of field and laboratory work, and checking data quality of reports. The overall objective of the system is to improve data quality for Cycle 2 EEM studies. The system is based on the ISO 14001 approach which includes the development and implementation of standard operating procedures for mills to adopt for overall EEM project management and the use of EEM data in environmental awareness training. The procedures include quality assurance and quality control checks which are integrated into existing Environmental Management Systems at mills.

BUILDING AND ASSESSING QA/QC OBJECTIVES/PROCEDURES APPLICABLE TO EEM CYCLE 2

G.P. Thomas and N. Munteanu. G3 Consulting Ltd., Richmond, BC.

Technical working groups, consisting of regulators, industry and scientists have reviewed data of Cycle 1 to provide insight and direction to Cycle 2 EEM programs and regulation amendments, by addressing site-specific factors while maintaining national data objectives. As a process, decision frameworks do not necessarily assess inherent data quality, which must still be evaluated by project scientists prior to, throughout and after use. Relevance and usefulness of Cycle 2 program data relies on data

integrity and relevant interpretations applied by project scientists as part of Cycle 1. Stringent QA/QC protocols and Standard Operating Procedures (SOPs) are utilized and emphasized for some EEM programs; however, industry standards applicable to field collections, data handling and storage and program regulation and enforcement have appeared to be less rigorous. Without sufficient audit at the data collection, manipulation, handling and reporting level, false data confidence may result. This paper discusses how data confidence and scrutiny should extend to all levels of environmental assessment and review. Concepts discussed include criteria and methods by which researchers may assess acceptability of data in Cycle 1 as a basis for Cycle 2 without compromising data or program objectives.

ENSURING EEM CYCLE 1 BASELINE DATA IS OPTIMALLY UTILIZED IN CYCLE 2

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Following a review of Cycle 1, technical working groups, consisting of regulators, industry and scientists have provided new decision processes for application to Cycle 2 EEM components, addressing site-specific factors while maintaining national data objectives. As tools, these decision frameworks do not necessarily assess inherent data quality, which remains to be evaluated by project scientists before further use. Relevance and usefulness of Cycle 2 program data relies on data integrity and relevant interpretations applied during Cycle 1. Given the original mandate of Cycle 1 work, application of this data to Cycle 2 may generate misleading conclusions based on a false confidence of data quality or objectives through nonjudicious use of tools. This paper discusses the principal criteria which must be achieved prior to utilizing any decision framework to ensure scientific integrity, including sufficiency and relevance of baseline information upon which Cycle 2 work is based and incorporation of ecological relevance by the integration of associated components (e.g., benthos, fish, etc.) or factors in the decision process to include scope for a 'weight-of-evidence' approach.

INTEGRATED CAGED BIVALVE METHODOLOGIES AS PART OF EEM: APPROACHES AND APPLICATIONS

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Understanding the utility of caged bivalves as part of EEM requires an understanding of: [1] the history of caged bivalve monitoring at pulp and paper mills in Canada; [2] approaches and applications used in other countries; and [3] the feasibility and scientific value of integrated caged bivalve methodologies for EEM. By 1960, caged oysters were used to monitor condition index and growth and by 1980, caged mussels were used to assess effects on growth and reproduction in marine waters adjacent to two coastal B.C. mills. Interestingly, while these two early studies only estimated effects by measuring bivalve growth in marine environments, several subsequent Canadian studies only estimated exposure to mill-associated chemicals by measuring bioaccumulation in tissues of caged freshwater bivalves. It is not surprising therefore that caged bivalve monitoring was included in early draft plans for EEM. It is not clear why this approach was excluded from the final EEM plan. By 1996, the first study integrating exposure and effects endpoints was conducted at a pulp and paper mill in Alaska as part of a U.S. EPA monitoring requirement. A number of refinements have been made to the methodology since 1960 and the use of bivalves as a monitoring tool is increasing. Recently, the use of caged bivalves has been established as part of an exposure-dose-response triad to support an integrated risk assessment strategy. A brief discussion and examples will be given of the several different approaches and applications of the method to support its use as part of EEM.

EVALUATION OF A MESOCOSM TECHNOLOGY FOR ENVIRONMENTAL EFFECTS MONITORING (EEM) METHODS APPLICATION

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Review of the Cycle 1, EEM Adult Fish Survey (AFS) identified several problems. Most mills did not capture the required number of sentinel species; there was a lack of understanding about the effluent exposure and migration history of the sentinel species; and when crustaceans were used as the sentinel species, they could not be aged properly. Alternative methods are needed at mill sites where the AFS approach was not appropriate. One of the alternate approaches which requires evaluation is the use of mesocosms. Mesocosms refer to field-based artificial stream systems which can be used in aquatic EEM programs as a tool to assess the effects of effluents on aquatic biota. Typically, the effects of effluents on aquatic receiving environments are assessed through field monitoring programs or effluent sublethal toxicity tests. Field monitoring programs can be inconclusive due to the high natural variability of sampled populations, inadequate reference sites for comparison to exposed sites, lack of understanding of the level of exposure, the presence of confounding effluent discharges, and/or inadequate statistical replication. The significance of sublethal toxicity test results can be difficult to determine as these tests lack realism, and in many cases use test species which are not endemic to the area being studied. Artificial streams can provide an integral link between field monitoring programs and sublethal toxicity tests by simulating the natural environment while allowing for variable control (i.e., sample sizes, sex ratios, effluent exposure), experimental manipulation, statistical replication, and providing cause-and-effect results to determine the effect of specific effluents on aquatic systems. The objective of this study is to evaluate if mesocosms are an acceptable alterative to the standard AFS.

BENTHIC SAMPLING STRATEGIES FOR EEM: CRITICAL EFFECT SIZES AND SAMPLING FREQUENCY

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A critical aspect of Environmental Effects Monitoring (EEM) for pulp and paper mills is the decision on the magnitude of an environmental impact that is deemed to be ecologically important. Determination of this critical effect size (ES_{crit}) is a prerequisite to study design and can play a key role in management decisions. Quantitative guidelines providing ES_{crit} values have not been widely developed for either marine or freshwater benthic assemblages. Some jurisdictions have, however, made initial attempts at setting ES_{crit} for benthic invertebrates, and some of these approaches may be applicable to the Canadian EEM Program. Most of these approaches have been at least partly based on departures from the normal range of variability in reference sites. One approach that could be used for the Canadian EEM is to set ES_{crit} equal to ±2 standard deviations (±2 SD) calculated from relatively nonimpacted reference communities. A useful modification of this approach is to determine standardized habitat-specific ES_{crit} values calculated by taking the medians of ±2 SD across several mills within given regions (e.g., for number of taxa, ES_{crit} = ±15% to ±60% relative to reference communities for different habitat types in the Pacific and Yukon region). Setting of criteria is likely to be an evolving process and the initial estimates of ES_{crit} will probably need to be revised as more data is collected during subsequent EEM cycles. A key recommendation for Cycle 2 and subsequent cycles is to increase the number of reference and impact stations (e.g., n = 5 or 8 for statistical power = 80 or 95%, respectively). Although these sample size recommendations are concerned with spatial replication, similar attention should be given to temporal replication. Of particular relevance is the issue of frequency of sampling. Early work suggests significant information loss when sampling on a three year rather than yearly cycle.

EEM - Transition to Cycle 2

EEM FISH SURVEY: A NATIONAL PERSPECTIVE AND TRANSITION TO CYCLE 2

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The Fish Survey Expert Working Group (FS EWG) reviewed the results of the adult fish survey of EEM Cycle 1 in order to identify technical difficulties and to recommend modifications or alternatives where existing approaches were inappropriate. The FS EWG assessed the variability of the measured parameters in order to allow proper statistical designs in subsequent cycles. Results of the variability analysis and evidence from other research were used to determine a target effect size of 20-30% for differences in gonad weights between exposure and reference fish. The variability analysis also gave insight as to the most appropriate capture methods and timing. Secondary objectives of the FS EWG were: [1] to determine the suitability and capture success of sentinel finfish species; [2] to evaluate the suitability of capture methods and gear; and [3] to asses the adequacy of reference sites. Recommendations for Cycle 2 were made in each of these categories, based on experiences in Cycle 1. A decision tree was developed to facilitate the transition to Cycle 2, and to aid regional officers, mills and consultants in determining the most appropriate study designs for Cycle 2.

THE USE OF CHEMICAL TRACERS TO MONITOR THE EXPOSURE OF INDIGENOUS FISH POPULATIONS TO PULP AND PAPER MILL EFFLUENTS

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In 1992, the Canadian federal government passed amendments to the Pulp and Paper Effluent Regulations (PPER) which included more stringent limits on biochemical oxygen demand, total suspended solids and acute toxicity to fish as well as a requirement for every mill to conduct an environmental effects monitoring (EEM) program. The objective of the EEM program was to determine if the fish, the fish habitat and the utilization of the fisheries resource were being adequately protected by the requirements of the PPER. During the first cycle of this EEM program, many pulp and paper mills experienced difficulty with meeting the requirement to analyze for effluent tracers in fish. The purpose of tracer analyses was to provide collaborating evidence of exposure of fish to the liquid phase of the effluent in the receiving environment. Most mills chose to measure resin and fatty acids in fish liver and bile while a few analyzed for chlorophenols, chloroguaiacols, or chlorinated dioxins and furans. In order to resolve some of the problems encountered with the fish tracer requirement, a small industry-government working group was established to examine the use and effectiveness of chemical tracers in assessing the exposure of indigenous fish to pulp mill effluents and to develop recommendations on tracer use for future pulp mill monitoring studies. Fish tracer results from the first cycle of EEM studies at about 76 Canadian mills with varied pulping and paper making processes and effluent treatment and discharging to different types of receiving environments were evaluated by the working group. The results of the evaluation indicated that while chemical tracers can be used to detect exposure at some sites, numerous factors greatly reduce their effective application at other mill sites. Furthermore, while verification of exposure of fish to effluent could generally be made, it is difficult to determine the extent of their exposure.

TAINTING EVALUATION RECOMMENDATIONS

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Six mills conducted fish tainting evaluations in Cycle 1 of the EEM program. In general, the results from Cycle 1 were inconclusive in that there were confounding factors which precluded the definite determination of whether or not the mill was responsible for flavour differences that were detected. Examples of the confounding factors were reference and exposure site selection, fish species selection, exposure regime and non-standardized sensory methods. The recommendations of the Usability of Resources (Tainting) Expert Working group (EWG) focused on standardization of the tainting evaluation protocol from initial experimental design and site selection to sensory evaluation and statistical treatment of the data. The recommendations of the EWG also included new wording, with accompanying guidance, of the tainting evaluation trigger.

DIOXINS AND FURANS RECOMMENDATIONS

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The Dioxin Expert Working Group (EWG) was established in the fall of 1996 to review the results of Cycle 1 dioxin sampling element of the EEM program. In the final report, the EWG reviewed the issues identified during Cycle 1 of the EEM program and made recommendations for future monitoring of dioxins to assess human-health concerns related to the consumption of contaminated fish. The EWG found that generally freshwater mills across Canada and marine mills in Atlantic Canada had low to non-detectable levels of dioxins in fish. However, some marine mills had high levels of dioxins in crab hepatopancreas and fish liver. Numerous problems were identified in the sampling, analyses and reporting of Cycle 1 data. To address these problems, the EWG has recommended clearer reporting requirements for the data, including QA/QC. As well, the following triggers are proposed by the Expert Working in future cycles: [1] levels of dioxins in the previous cycle were below the consumption guidelines or there is no consumption guideline-related advisory/closure based on dioxin/furan contamination for any species or tissue in the receiving environment of the mill; and [2] the mill has been in compliance with the CEPA regulations for dioxins and furans in final mill effluent for the preceding 12 months. At sites where the levels of dioxins reported are approaching the advisory guideline (e.g. within 30%), and there are no other supporting data or programs, the EWG suggests that the Regional Authorization Officers may require that dioxin analysis be included in the next cycle of EEM.

RECOMMENDATIONS FOR THE BENTHIC COMMUNITY STRUCTURE COMPONENT OF EEM CYCLE 2 FOR THE PULP AND PAPER INDUSTRY

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The results of the Cycle 1 benthic invertebrate community survey which was carried out as part of the Environmental Effects Monitoring (EEM) program for the pulp and paper industry were reviewed to assist in a second cycle. The Benthic Community Expert Working Group (BC EWG) based its review on the proceedings of various government/industry regional workshops, summary spreadsheets prepared by Environment Canada, the Cycle 1 experience of the BC EWG members and summary reports prepared for the Atlantic, Quebec and Ontario regions. Identified as major issues for review were, the ability to relate invertebrate community response to mill discharges, the standardization of sampling methods and the suitability of methods for data analysis. Of the mills reviewed (112), 69% of the studies showed differences in species richness and/or abundance between reference and exposure sites, although fewer (28%) studies suggest that the differences were effluent-related. The interpretation of the remainder was confounded by factors such as multiple discharges and reference site selection. A variety of sampling methods were used in Cycle 1 including different devices, mesh sizes and subsampling techniques. This lack of standardization made it difficult to carry out a national or regional assessment of the environmental performance of mills. The data analysis requirements were overly complex and restrictive in Cycle 1. The use of multivariate analyses was inappropriate for the study design and, in many cases, consultants derived their conclusions based on simple metrics rather than the results of the statistical analyses. In addressing recommendations for Cycle 2, the BC EWG considered the first cycle issues as being related to problems with: [1] study design and site selection; [2] sampling methodology; or [3] interpretation. Within 'interpretation', several sub-categories were identified including supporting field measurements, data measurement variables and analysis, data handling and screening, and effect size. With respect to effect size, no agreement was reached on what constitutes and 'effect'. Finally a decision tree was developed to assist in planning studies for Cycle 2.

MAJOR EEM CYCLE 1 FUNDINGS AND RECOMMENDATIONS ON LABORATORY TOXICOLOGY FOR CYCLE 2

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With the completion of Cycle 1 monitoring requirements under the pulp and paper EEM program, there was a need to examine the results of the laboratory toxicology testing and to suggest adjustments to the requirements to improve the quality and value of the program. A fourteen- person industry/government expert working group (EWG) on laboratory toxicology was formed to assess the Cycle 1 data and provide Cycle 2 recommendations. From July 1996 to April 1997, the EWG met on seven occasions to: [1] discuss issues identified by industry/government workshops and the

environmental consultants who completed the testing during Cycle 1; [2] the ability of Cycle 1 tests to detect effluent quality changes; [3] possible relationships between the laboratory sublethal toxicity data and observed field effects; [4] potential additional or alternative tests; and [5] the frequency of sublethal testing for Cycle 2. Overall, the EWG felt that Cycle 1 sublethal toxicity testing was successfully completed and recommended that it should remain part of the core program for Cycle 2. It was recognised that installation of secondary treatment lead to a significant improvement in effluent quality with respect to sublethal toxicity. Recommendations have been made related to refinements to specific test methods, Annex 1 changes to strengthen the quality assurance aspects, frequency of testing for Cycle 2, changes to Environment Canada's interpretive guidance and the need for further methodology research and method validation. The presentation will focus on the specific recommendations of the EWG.

EEM INFORMATION MANAGEMENT

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The Environmental Effects Monitoring (EEM) Program data includes physiochemical, toxicological, biological and other site-specific parameters, spanning several sampling seasons per cycle. This data will be reported electronically to Environment Canada. As the data are received by Environment Canada they will be stored in a data base. Access to this data base will be via the Internet. The data base will assist in trend analysis, comparative analysis, statistical analysis and correlation of the data.

EEM CAGED MUSSEL PILOT STUDY AT THE PORT ALICE SULFATE MILL, VANCOUVER ISLAND, BC

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Environment Canada conducted an *in-situ* caged mussel EEM pilot study to evaluate this approach and to address issues that have been raised regarding applicability at Canadian mills. The objective of this study at the Port Alice sulfite mill, Vancouver Island, B.C., was to test the feasibility, scientific value, and applicability of using caged bivalves as a tool for EEM. Effects endpoints included survival, growth, percent water, and percent lipids. Exposure endpoints included mussel tissue chemistry and chemical analysis of lipid bags (semi-permeable membrane devices; SPMDs). To provide the option of including this methodology as part of Cycle 2 of EEM, the pilot study was conducted between August-October, 1997, so the final report would be completed by December 31, 1997. Planning has been open to industry, government, and consultants. Representatives from each group also observed and participated in the sorting, measurement and deployment, and retrieval processes. The scope of work included technology transfer elements for a combined Pilot Study-Workshop using caged mussels. The study will be evaluated based on the criteria established in Annex 1 of EEM: [1] scientifically defensible; [2] cost-effective; [3] flexible to incorporate new or improved methodologies; [4] evolutionary to build on relevant ongoing research; [5] manageable with respect to requirements and timeframes; [6] generation of interpretable results; [7] incorporation of a weight-of-evidence approach to interpret results with respect to effects; and [8] use of well-defined decision points. A description of the pilot study and some preliminary results will be presented.

ENVIRONMNETAL EFFECTS MONITORING: THE ROLE OF RESEARCH AND NEXT STEPS

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The Environmental Effects Monitoring (EEM) program for the pulp and paper industry is a sequential series of monitoring and interpretation cycles designed specifically to assess the adequacy of the Pulp and Paper Effluent Regulations (PPER) of the federal Fisheries Act (1992). The design and evolution of the EEM program is based on several key principles. The program must: [1] be scientifically defensible; [2] allow for flexibility to accommodate site-specific requirements; [3] be cost-effective; [4] be flexible so that new or improved monitoring techniques can be incorporated; [5] be capable of building on findings of relevant research programs; [6] be manageable with respect to its requirements and timeframes; [7] generate interpretable results; [8] use a 'weight of evidence' approach to interpret results with respect to effects; and [9] provide defined decision points. Hence, as EEM is based fundamentally on a scientifically credible program and process, its future evolution and improvement necessitates the consideration of the role of research.

Under the national government-industry review of Cycle 1, six scientific Expert Working Groups (EWGs -Tracers, Dioxins, Usability of Resources, Fish, Benthic, and Toxicology) established under the Technical Management Committee (TMC), provided recommendations on how to improve the scientific basis of subsequent EEM cycles. Each of the EWGs identified both the positive aspects and shortcomings of the present EEM design for each of their subject areas. The TMC considered these recommendations in revising the scientific guidance provided in the Annex to the regulation and associated Technical Guidance Documents for implementation in Cycle 2. This review process allowed for the identification of critical information gaps, poor or inappropriate methodologies, inadequacies in statistical and field program designs, and potential alternative approaches. It was also identified that many of these shortcomings required further research before improvements or alternatives could be implemented in an EEM program.

Building on the EWG reviews and recommendations, the TMC has initiated the organization of a research workshop in December 1997 that will be used as a first step to identify present and emerging research issues and priorities for the EEM program. The workshop stems from the recognition that EEM should be flexible and build on existing and future relevant national and international research initiatives. The TMC also recognized the importance of coordinating research efforts where possible so that joint government / industry priorities can be addressed. Hence, national research or pilot studies could be designed that are targeted at improving EEM through the development and validation of new, cost- and information-effective analytical / monitoring techniques. However, appropriate screening and decision criteria must also be developed that would be used to determine which and whether of these new or alternate approaches should be incorporated into a present or future EEM cycles.

WRAP UP AND CYCLE 2 REQUIREMENTS

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The finalized Annex 1 document was developed through a consensus-driven process by committees made up of equal representation from both government and industry. As a result of a shared commitment by both groups to improve the overall EEM program, agreement was reached in all major areas of the EEM program. Key steps in generating the revised Annex 1 included the completion of the Expert Working Group (EWG) reports, the review and prioritization of the EWG recommendations by the Technical Management Committee (TMC), the detailed reviews of draft Annex 1 documentation by members of the TMC and the ability to have outstanding issues resolved by a Management Steering Committee. The redesigned EEM program is more flexible than Cycle 1 and, as a result of the inclusion of comprehensive decision trees, the program will allow for site-specificity including the ability to modify or suspend core requirements of the EEM program, if scientifically justified at a given site. Where possible, effect sizes have been provided to assist in interpretation of results. Improvements have also been made in the areas of statistical analysis and sampling requirements. Work still needs to be done in determining alternative methodologies for various program elements, in identifying realistic effect sizes and in understanding natural variability.

Pollution Prevention

POLLUTION PREVENTION AND TOXIC SUBSTANCES

S. Forbes. Canadian Centre for Pollution Prevention, Sarnia, ON.

The elimination and management of toxic substances is a strategic issue for the protection of water systems. This presentation will describe what pollution prevention is, how pollution prevention planning is becoming an important environmental tool, and how it has been applied to aquatic systems in the Great Lakes region. The presentation will describe the drives for change and their impact on the aquatic environment: [1] non-regulatory policies and programs, how and when they should be used; [2] accounting for the environment, and the impact on the bottom line; [3] product Lifecycle Analysis and the changes in manufacturing; [3] watershed and ecosystem management; and [4] information systems available to support informed decisions. Practical examples from Ontario pollution prevention projects will be used to demonstrate that it is an effective strategy but one that requires new thinking, new approaches and new technologies.

THE ANATOMY OF A MANURE SPILL

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This paper reviews data on manure spills to surface waters over the past nine years (1988 - 1996, inclusive). This analysis was undertaken as a goal of the Livestock Manure Pollution Prevention Pilot Project. By analyzing Ontario Ministry of Environment and Energy (MOEE) Spills Action Centre (SAC) information on manure spills, it was hoped to better understand the anatomy of a manure spill and in turn to be better able to make recommendations designed to reduce the likelihood of spills and their subsequent impacts. The impacts of manure spills include bacterial contamination, oxygen depletion, nutrient enrichment promoting excessive plant growth and subsequent disruption of the oxygen regime, toxicity to aquatic life and destruction of fish habitat. With the trend towards larger livestock operations, the challenge of adequately controlling larger volumes of manure, will also grow. An initial analysis of provincial data revealed that most manure spills took place in the MOEE Southwestern Region (Orillia to Windsor to Woodstock). Subsequent, more detailed analysis of spill scenario details, focused on the Southwestern Region statistics. Although data was not consistent or comprehensive throughout, trends and common elements were obvious. Findings reflect both the adequacy of manure handling and the adequacy of manure spills information gathering. In conclusion, most manure spills over the past nine years have taken place in Southwestern Ontario. Almost all have involved liquid manure, mostly swine, and the majority have been the result of manure spreading by spray irrigation with manure gaining access to watercourses through tile drains. Spill occurrence details have been documented in varying degrees of completeness by the existing MOEE reporting system. An important conclusion of this statistical analysis draws from the realization that a more comprehensive and consistent reporting system is needed. This can hopefully be easily achieved through further refinement of the existing system.

REDUCTIONS IN VOLATILE ORGANIC COMPOUNDS FROM AUTOMOTIVE REFINISH INDUSTRY

J. Norris. Hamilton District Autobody Repair Association, Hamilton, ON.

The 2600 collision repair/auto refinish shops in Ontario are believed to represent 21% of all VOC emissions from surface coating in Canada. Although each shop may be a small emitter, together the industry generates a significant level of VOCs. In comparison, the automotive original equipment manufacturers generate 25% of all VOCs from surface coating operations, but paint up to 40,000 complete cars a week and use up to 4 times more paint than all refinish shops combined. The automotive refinish industry, the coatings companies and equipment suppliers, have been involved for over two years in dsicussions leading to the package of recommendations waiting for the approval of the Canadian Council of Ministers of Environment. Those recommendations will reduce emissions from auto refinish shops by almost 50% and are an innovative example of how an industry-driven program in this era of government resource difficulties, can provide high levels of compliance to strict standards while delivering built-in benefits for the shop. The recommendations involve VOC content paint changes, paint spray gun efficiency improvements, possession of an approved spraybooth and waste management agreement and mandatory training. 80% of all income into the industry comes from insurance companies, who are funding the painting of a collision-damaged vehicle. It is proposed that only shops that meet these new enivronmental standards will be eligible for auto repair settlement cheques from insurers.

EVALUATING AND DEALING WITH GREENHOUSE EFFLUENT

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The purpose of this ongoing study is to identify and quantify fertilizer effluent from greenhouses into nearby ponds and ditches form which water may be drawn for irrigation. There is a perception that greenhouse runoff is a potential environmental problem. Our results to date have shown that there are difference in ion concentrations between clean margin ponds and vegetated ponds. For example, nitrate levels in excess of the ground water threshold were seen in clean margin ponds while vegetated ponds exhibited levels lower than the threshold. Most ions were not found in excess of the ground water threshold, however, levels of 80 mg/L chloride and 65 mg/L phosphate were often evident in certain ponds. These levels could be detrimental to crops and may contribute to accumulation in a recirculating system if the water is used for irrigation. Techniques such as recirculation of the nutrient solution and filtration of the solution can be used to reduce runoff to the environment. The Ontario greenhouse industry is studying and applying these techniques. The solutions must however, be cost effective and still allow producers to maintain superior quality production. Clearly, recirculation of the nutrient solution is a very effective method of mitigation the environmental impact of greenhouse runoff. Unfortunately, there are serious consequences to long term recirculation including; limitations of sensor techniques causing ionic imbalances, salt accumulations such as chloride and sulphate, and pathogen proliferation. Current data suggest that greenhouse effluent is not a problem at this time.

POLLUTION PREVENTIOM IN THE U.S. PULP AND PAPER INDUSTRY

T.L. Deadorff. International Paper, Loveland, OH.

The U.S. pulp and paper industry is committed to constant environmental improvement. We are continuously looking for better methods and to adapting technologies to achieve this goal. Concerning the impacts of our effluents on adjacent aquatic ecosystems, several significant advances are noteworthy, such as our: [1] voluntary and rapid program to eliminate dioxin discharges; [2] nationwide implementation of secondary biological treatment; [3] trend toward reduction of the use of chlorine in bleaching; and [4] use of elemental-chlorine-free (ECF) bleaching. As part of our continuing efforts to understand the potential impact of biologically treated effluents to the aquatic ecosystem, our companies have conducted integrated field studies on various receiving streams, which included comprehensive water and sediment toxicity testing, benthic community evaluations, fish health testing, and physicochemical and biological parameters of water quality. To date, no indications of detrimental impacts to the ecosystem from the effluent have been found in U.S. mills using ECF technology. These and other issues will be presented.

POLLUTION PREVENTION AND SMALL TO MEDIUM SIZED PRINTING ENTERPRISES

J. Farmer. Printing and Graphics Pollution Prevention Project, Owen Sound, ON.

Aquatic toxicity is very important to Canada and Ontario. Twenty percent of the world's drinkable fresh water is contained in the five Great Lakes. Almost all of the sewers in Ontario eventually discharge to this watershed. How we manage our stewardship of this resource must be based on pollution prevention; not on end of pipe controls, or spill response. Prevention is the key. The P2 for Printers Steering Committee was charged by the federal government, the provincial government and industry with encouraging pollution prevention in the printing industry. We agreed that small and medium sized enterprises (SME's) needed help more than large companies who in general already had environmental programs in place. In developing an approach much thought was given to why SME's were not practicing P2. It came down to: information, motivation and communication. Information on compliance and best management practices was not readily available in a clear concise form. There was no motivation to go beyond compliance, and with cut backs to government enforcement programs even compliance was not seen as a requirement. The need for an Environmental Management System that ensured compliance, due diligence and reduced costs was not being communicated. A self audit checksheet/guidebook was developed, and is being distributed through a series of twelve, province wide workshops entitle 'Less Waste...More Profit'. These workshops are partially sponsored by industry, and also have a cost recovery component. Primary funding is from the federal and provincial governments. The project has shown that great strides can be made in pollution prevention if the information is available, there is a motivation to adopt best management practices, and the information and benefits are communicated to the SME's. However in a fragmented industry such as printing, government financial assistance is required to make it happen.

BLUEPRINT FOR MERCURY ELIMINATION: POLLUTION PREVENTION CASE STUDIES

J. Harvie. Duluth, MN.

The Western Lake Superior Sanitary District (WLSSD) is a wastewater treatment facility created in 1971 to minimize pollution in the St. Louis River. It is the largest point source discharger on the U.S. side of Lake Superior. Under its current discharge permit the WLSSD must meet an effluent mercury limit of 0.030 µg/L. New requirements proposed under the Great Lakes Initiative will require even more stringent limits for mercury. The WLSSD has embraced pollution prevention as the most effective tool to meet this challenge. This discussion will focus on nationally recognized mercury pollution prevention initiatives with the pulp and paper industry, the healthcare industry and the University of Minnesota - Duluth, College of Science and Engineering.

THE AQUATIC ENVIRONMENTAL IMPLICATION OF EMERGING METALLURGICAL TECHNOLOGIES

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Traditional emerging metallurgical processes are significant source of water pollution. Two types of contamination can be determined as exhausting after traditional 'wet' metallurgical processes: chemically passive, which presents the suspension of micro and submicro metal powder in a water, and chemically active, which presents the week electrolyte, containing metal ions.

Many large scale volumetric metallurgical processes such as ore extraction, metal recycling containing row materials and heat treatment use water as a mass and heat transfer media for separating, extraction, heat exchange and cleaning/ washing purposes. Surface engineering processes (nitriding, boriding, carburising, coating deposition) use water for preoperation and postoperation treatment of metal surface and technological media as well in specific technological processes such as electroplating. Traditional processes using with salt baths result in water release containing cyanides traces. Another wide distributed technology- electroplated chrome - gives rise to water contamination release by traces of electrolyte. Chemically active hexavelant electroplated chrome can be released from the final product over a prolonged period.

Behavior of contaminants in aquatic systems strongly depends on physical-chemical properties of both a contaminant and the environment (BIOMOVS, 1996). Special study indicates the following forms of contaminant in the system 'water-bottom deposition': dissolved cations; exchangeable forms, including radionuclides absorbed onto soil or bottom sediments by ion exchange mechanisms; non-exchangeable forms, including radionuclides of nuclear fuel particles and fixed radionuclides on mineral or organic components of soil or bottom sediments. Countermeasures, after accidental release, should take into account the distribution of these forms in aquatic systems. For example, adding clean water to input of aquatic system in order to contaminated water dissolving and decreasing of contaminant concentration results in concentration increasing in the outlet. An example of active contaminant dispersion and water quality managing after accidental release is described in Slavik et al. (1997).

The novel 'dry' metallurgical processes based on plasma and vacuum technologies are replacing existing 'wet' processes. They reduce release of chemically passive contamination and practically cancel release of chemically active contamination. The potentials of plasma metallurgical processes can be illustrated by several examples. Using 'dry' plasma-ion cleaning instead washing provides absence of chemically active contamination and reduce releases of technological water up to 50%. Using CVD, PVD, ion nitriding, ion carburising and Plasma Source Ion Implantation (PSII) technologies exclude entirely any chemically active contamination. Plasma coating technologies, ion nitriding or PSII process are an alternative for electroplated chrome and thus exclude release of electrolyte and hexavelant chrome. The replacement of hexavelant chrome with trivalent chrome by traditional electroplating has met with very limited success (Barnard).

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Risk Assessment

THE ROLE OF SCIENCE IN RISK MANAGEMENT DECISION-MAKING

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The risk assessment/management paradigm currently favoured by the U.S. EPA insists on the separation of assessment and management functions without effectively defining the domain of either. European and Canadian approaches have followed more traditional approaches to environmental management by including recognition of the importance of stating decision criteria and the influence of societal values on the conduct of environmental policy. Both implicitly adopt frameworks which facilitate making the distinction between science and science-based policy through the use of *a priori* decision criteria. In the context of risk assessment decision criteria are equivalent to the selection of endpoints and threshold values. They determine the types of information to be gathered, and when thresholds act as action triggers in the decision-making process. Decision criteria are, however, selected based on societal values which may include science-based concerns. Thus, risk assessment alone is not a complete or socially useful framework for the development of environmental policy. Completeness requires that risk assessment be practised within a well-defined and societally-accepted decision-making framework where the rationale for actions, scientific or other, are clearly understood. Only then will risk assessment live up to its potential as a valuable conceptual and methodological extension of traditional environmental impact assessment techniques. Examples comparing and contrasting risk assessment and decision-making frameworks and practices will be discussed to illustrate the issues raised above.

USING BODY BURDEN CONCENTRATIONS FOR RISK ASSESSMENT OF METALS IN THE ENVIRONMENT

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Traditional methods of assessing the risk of toxic chemicals in the environment suffer from two drawbacks. They do not readily allow identification of the toxic agent, and they do not provide an early warning system. Most comprehensive environmental assessments rely on a three-pronged approach: chemical analyses, toxicity testing, and examination of natural populations. Toxicity testing and examination of natural populations may demonstrate an effect, but only if the damage has already occurred. Furthermore, the toxic agent is not identified. Chemical analyses may provide clues as to what the toxic agent is, but the uptake and toxicity of many contaminants is strongly affected by binding to dissolved and particulate matter in the environment and interactions between the organism, toxicant, and environmental chemistry. This makes it extremely difficult to predict toxicant effects from environmental concentrations. These problems can often be overcome by examination of body concentrations, because biological effects usually occur as a result of bio-accumulation of the toxicant. Unfortunately, the relationship between body concentrations and effects has received much less attention that the relationship between toxicity and water or sediment concentrations, largely as a result of our preoccupation with water or sediment quality criteria. Furthermore, since toxicant bioaccumulation can often be detected before biological effects occur, body concentrations can provide an early warning of impending problems. Some examples of how tissue analyses can be used in risk assessment of metals will be presented.

A COMPARISON OF CAGED AND NATIVE MUSSELS (*Elliptio complanata*) AS BIOMONITORS OF ORGANIC CONTAMINANTS IN THE ST. LAWRENCE RIVER

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Freshwater mussels (*Elliptio complanata*) from Balsam Lake, ON, were caged for 12 weeks at 3 sites in the St. Lawrence River Area of Concern. Upon retrieval of cages, native mussels of the same species were also collected. Fifteen caged and 15 native mussels from each site, plus 15 pre-exposure animals, were individually analyzed for residues of organochlorine pesticides and PCBs. Caged mussels adjusted their body burdens of Σchlordane, Σendosulfan and ΣPCBs to resemble those in native mussels from the same sites; e.g., Σchlordane decreased from a mean of 1.4 μg/kg dry weight in mussels from Balsam Lake to 0.2-0.3 in caged vs. 0.2-0.5 in native mussels, and ΣPCBs increased from 37 μg/kg to 108-337 in caged vs. 115-535 in native mussels. In contrast, caged mussels accumulated very little mirex from the 2 sites where it occurred in native mussels. Concentrations of Σaldrin, ΣBHC, Σchlorobenzene and ΣDDT did not vary significantly among Balsam Lake, caged or native mussels. Both caged and native mussels showed that differences in contamination among the 3 study sites were minimal for all compounds except PCBs. Numbers of PCB congeners were lowest in mussels from Balsam Lake (25), and numbers were significantly lower in caged than native mussels at all sites (44, 57 and 60 vs. 55, 67 and 72, respectively). Numbers of congeners in native mussels differed significantly among sites, but there were no differences between 2 of the sites for caged mussels. Concentrations of ΣPCBs were significantly lower in caged than native mussels at the most contaminated site. Results suggest that caged mussels may be useful biomonitors of organic contaminants in the St. Lawrence River, but that 12 weeks exposure may not be sufficient for discerning trends for some compounds.

PREDICTIVE ASSESSMENT OF OIL SPILL IMPACTS ON AQUATIC BIOTA

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Oil spill impacts on aquatic life are often estimated using chemical fate and transport models to calculate organism exposure, dissolved aromatic toxicity models to calculate organism mortality, and simple production foregone or adult equivalent models to calculate population effects beyond the initial kill. The assumptions and databases involved in these calculations are critically reviewed, some data gaps and sources of uncertainty are identified, and the consequences of this uncertainty are discussed. It is suggested that uncertainties in practice are often very large and that the modelling estimates of biological injury, without strong supporting empirical evidence, may not provide an adequate basis for assessment of environmental damages.

CONTAMINANT TRANSPORT MODELLING AND RISK ASSESSMENT FOR A NICKEL-COPPER-COBALT MINE MILL FACILITY

C. Russell, N. Morris, D. Lush and D. McMillan. BEAK International Inc., Brampton, ON.

The development of natural resources, such as minerals, oil and gas, forestry products, etc., often results in the modification of the local environment and consequent changes to nearby aquatic and terrestrial habitats. In the evaluation of the environmental significance of such changes, it is necessary to take an integrated ecosystem level approach. One important aspect of this approach involves being able to quantify the degree and nature of habitat alteration resulting from chemical emissions and their effects at a number of possible sites, and to evaluate their significance in both a local and more regional context. Given the fact that effects of chemical releases on ecosystems may result from a number of sources such as air emissions, aqueous surface water discharges, groundwater seepage, soil erosion, accidents and spills, and also may involve a broad range of organic and inorganic chemicals, the tracking and quantification of these chemicals in environmental media can become a complex task. In order to assist in this type of evaluation, BEAK has developed a series of integrated probabilistic environmental risk assessment models managed under a master software program called IMPACT. The software uses a geographical information system interface in order to better communicate to a technical and public audience a number of complex environmental risk variables that fluctuate in space and time over a number of project phases (e.g., development, operational and decommissioning). The model incorporates the emissions of trace elements and organic materials to atmospheric, groundwater and surface water transport pathways, and mathematically simulates the transport and fate of these materials in physical and biological systems over space and time. Accordingly, it allows analyses such as the evaluation of the effect of atmospheric emissions on aquatic systems during an operational phase, or the effects of operational groundwater seepage on site biota in a decommissioning phase. This paper presents a case study of the application of the model at a mine site and illustrates some of the types of non-intuitive issues that can be forecast to occur and how this type of forecasting can be used in the design of appropriate mitigation and monitoring programs.

ECOTOXICOLOGICAL EVALUATION OF DUST ABATEMENT PRODUCTS

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Since 1996 Technitrol-Eco has completed 4 risk evaluation of dust abatement products using BNQ prenorm NQ 2410-300. This type of assessment aims at evaluating the level of risk associated with the used of dust abatement products. The assessment involves the assigment of a notation for the following ecotoxicological considerations: 'origin', 'fate' and 'effect'. This is completed using the following eight parameters of evaluations: [1] level of chemical complexity; [2] presence of critical contaminant; [3] degradability of the product; [4] mobility of the product; [5] bioconcentration of critical contaminants; [6] level of aquatic toxicity; [7] level of terrestrial toxicity; and [8] level of genotoxicity. Marked differences were observed with the four products tested and more specifically in regards to the level of degradability. Two products met the prenorm requirements while a third one failed after completing battery of toxicity testing. A fourth product evaluation was interrupted after completion of the chemical analysis due to the presence of a large number of unwanted chemicals. Modifications to the pre-norm had to be instituted in order to allow testing of products with low toxicity and/or water solubility. In conclusion, the prenorm was shown to be applicable in a laboratory setting. However, more flexibility is required for the testing of products with low toxicity or water solubility. The proposed approach for the evaluation of the genotoxic potential should also include a confirmation step.

ASSESSMENT OF HUMAN HEALTH RISKS FROM A LIGHT CONDENSATE PLUME MIGRATING TOWARDS A RURAL RESIDENTIAL DEVELOPMENT

T.L. Knafla and J.H. Sevigny. Komex International Ltd., Calgary, AB.

A light condensate and dissolved phase plume, originating from a small gas plant, has migrated downgradient of a site in rural Alberta, towards a residential development. Target and non-target gas chromatography/mass spectrometry analyses were conducted on condensate and on critical transport media (i.e., groundwater and soil air). These analyses identified predominantly linear and cyclic alkanes (alkanes) and minor amounts of monocyclic aromatics. A greater relative fraction of monocyclic aromatics was found in groundwater and only alkanes were detected in soil air. These result were consistent with theoretical predictions. A deterministic screening exercise was conducted to evaluate risk from subsurface vapour migration and domestic use of groundwater (e.g., ingestion, showering). The screening exercise resulted in the following conclusions: [1] a risk management strategy should be implemented to prevent the condensate from reaching residences; [2] residents should not use groundwater for domestic purposes; and [3] the concentration of compounds in the dissolved phase should be restricted. A probabilistic assessment was conducted to established remediation objectives for the dissolved phase plume via subsurface vapour migration. The results were significant because they illustrated that alkanes, and not benzene, pose the greatest potential health risk. Alkanes have significantly greater Henry's law constants and yield much greater subsurface vapour concentrations. The major implication of these results is that regulatory criteria established for protecting health usually focus on BTEX compounds. In certain situations (as in the current case), alkanes present a much greater health risk. The exclusion of alkanes from a list of regulated target compounds may compromise human safety.

Statistical Applications

THE USE OF SAFETY (UNCERTAINTY) FACTORS IN TOXICOLOGY AND RISK ASSESSMENT

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Uncertainty is part of risk assessment. For instance, only laboratory data may be available, field/epidemiological data may be limited and less than clear cut, and so on. There are various means to deal with uncertainty; however, the most common is the use of simple 'safety' or 'uncertainty' factors. These remain relatively little changed since their origin in 1945 and are presently used to extrapolate, for both aquatic and terrestrial environments: inter- and intra-species, acute to chronic, LOEC to NOEC, laboratory to field (e.g., extrapolation of laboratory results to a no-effect concentration in the field). Such extrapolations do not always have a clear relationship with the field effect(s) of concern, nor are they always based on good science. Realistically, safety factors will not be readily replaced and they have their uses. But they are presently, too often, abused. For instance, the so-called 'precautionary principle,' which originated in 1980, effectively addresses risk by proposing that the safety factor should be infinitely large. Suggested improvements to current usage include: providing safety factors as a potential threshold effects range not as a discrete number; and, using experimental results, however uncertain, rather than defaulting to safety factors to compensate for lack of information. This latter recommendation has the additional value of rendering safety factors predictive rather than simply protective.

APPLICATION OF META-ANALYSIS TO EVALUATE DATA FROM INDEPENDENT TOXICITY STUDIES

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Meta-analysis is the use of formal statistical techniques to synthesize the results of separate but similar experiments. It works by combining information from various studies in a way that accounts for different scales of measurement of response variables, the magnitude of the effect observed, and the sample size. These techniques, which have been applied to research in the social sciences, medicine and ecology, should be useful for quantitatively reviewing data on the toxicological effects of contaminants. Meta-analysis is particularly effective where: [1] results vary across studies; [2] the expected magnitude of an effect is small, and [3] sample sizes of individual studies are low but the number of available studies is high. Drawing on a potentially broad range of properly selected studies, meta-analysis can address questions such as: [1] does a specific concentration of a compound (e.g. NOEC, MATC, regulatory standards) produce a toxicological effect; [2] Does a certain environmental disturbance (e.g., discharge of metal contaminants) alter the receiving biotic community (e.g., decrease total biomass); and [3] do any factors in the studies influence the magnitude of the effects. An example of an application of meta-analysis to a series of toxicity tests results will be given. Advantages and disadvantages of the approach will be discussed.

ASSESSING BIOLOGICAL EFFECTS WITH NON-CENTRAL TESTS

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Environmental monitoring programs typically measure some aspect of the ecosystem worth protecting. Data from these types of monitoring programs are often used to compare presumably impacted locations with unimpacted reference locations. Regardless of the particular experimental design, statistically significant differences in response between the test location and the reference locations are often used as evidence of a significant impact. Usually these tests involve an analysis of variance or some nonparametric equivalent. A major weakness in this traditional approach is that statistical significance can be guaranteed if enough samples are collected, irrespective of the size of the impact. In order to avoid this problem, equivalence tests should be used to test null hypotheses which specify non-zero effect sizes. Thus our null hypothesis becomes: H_O: the difference is ≥ our critical effect size. The equivalence test uses a non-central distribution to establish the probability that the observed effect is equal to, or larger than, the critical effect size. If the effect is truly larger than the critical effect size, then conclusions based on the equivalence test will be incorrect 5% of the time. If the effect is truly smaller than the critical effect size, then small sample sizes could erroneously lead us to accept H_O, thereby concluding that an impact had occurred. Increasing the number of samples will ensure that we correctly conclude there is no impact (i.e., in order to reject H_O). This feature of non-central equivalence tests encourages proponents to invest in collecting sufficient samples to prove their innocence. Moreover, this approach eliminates the need to debate the minimum number of samples necessary to ensure that environmental effects monitoring programs protect the environment.

ENVIRONMENTAL MONITORING PROGRAM DESIGN

M.D. Paine. Paine, Ledge and Associates, North Vancouver, BC.

Monitoring programs consist of three components: variables, locations and times. Most programs measure too many variables and consequently monitor too few locations or times. With a fixed budget, each variable exacts a cost in terms of the numbers of location or times which can be monitored. The power of statistical tests depends on the number of locations or times, and not on the number of variables. Therefore, the common misperception that "the more variables you measure, the more you know about the study system" impairs the effectiveness of environmental monitoring. Reports and RFPs should provide the maximum number of variables to be measured, not the minimum. Spatial replication should be on a large scale; subsampling within larger spatial replicates is rarely cost-effective unless the subsamples are composited. Replicates should be randomly selected. Divide the area to be sampled into smaller units, with the number of units 2-3 times the number of replicates required. Randomly select the units to be sampled, thoroughly subsample within each, then composite the subsamples into a single sample per replicate. The optimal design for impact assessment of point sources is not to sample intensively around the source, but to sample many references. The point source area is then treated as a single observation to be compared with the sample of references. Optimizing temporal replication over long terms is much more difficult. Selection of sample years is limited by the timing and duration of the program. There is no way to select similar sample years *a priori*. In an impact assessment, baseline monitoring will be limited to one or a few years immediately before start-up, even though sampling more baseline years over a longer period would be better.

DEFINING THE WORD 'REPLICATE' IN THE CONTEXT OF SAMPLING BENTHIC MACROINVERTEBRATE COMMUNITIES

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When sampling benthic macroinvertebrate communities, multiple grabs are usually taken in close proximity, to enumerate the community in a given area. These grabs are often defined as replicates. In the case of a benthic macroinvertebrate community where aggregation occurs, samples collected close to one another are not independent. These grabs are more appropriately termed subsamples and not replicates. The definition of replicate is "the smallest unit to which an experimental treatment may be applied." In the case of sampling benthic macroinvertebrate communities, replicates are those areas sampled far enough apart to be uncorrelated but still within the general area of the experimental treatment, often an effluent, and subject to the same confounding variables. Thus "control-impact" surveys with n_c control sites and n_i impact sites with s grabs at each site are often described as having $(n_c + n_i) * s$ samples when in reality only $n_c + n_i$ samples have been collected, albeit with s subsamples. This has important implications when designing benthic macroinvertebrate surveys.

DATA ANALYSIS IN BENTHIC INVERTEBRATE STUDIES: SEARCHING FOR SOUND, SIMPLE AND SENSITIVE STATISTICS

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Benthic Invertebrate studies of all kinds frequently suffer from coarse and inadequate analysis and interpretation in which subtle trends are likely to be missed. Conversely, data may be subjected to complex numerical procedures that are difficult for non-specialists to apply or assess and which sometimes allow statistics to dominate over biology. As an ordinary biologist trying to understand how invertebrate communities respond to disturbance, I advocate an emerging protocol for data analysis emphasizing: [1] statistical analysis founded on simple, established, hypothesis-testing methods; [2] careful selection of appropriate and meaningful variables for analysis; [3] use of statistics to guide interpretation of results in conjunction with knowledge of the biology of the animals; [4] optimized sampling and collection of physical and habitat data at each site to help reduce extraneous variability; [5] use of multivariate descriptive statistics (ordinations, clustering) only as a preliminary tool to identify key trends or taxonomic groups; [6] weight-of-evidence reasoning based on parallel analysis of several taxa or variables to determine whether and to what degree the benthos has been affected and strong inference to implicate the disturbance source; and [7] simple, straight-forward data presentation comprehensible to the non-statistician. The protocol has been developed based on literature recommendations and personal experience and tested largely in prairie and western rivers. Further refinements are still needed.

Science in Regulation Development

NEW DIAGNOSTIC TOOLS FOR WATER QUALITY MANAGEMENT IN DEVELOPING COUNTRIES

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A dilemma in many developing countries is the need to rapidly assess the nature and source of toxicity in aquatic systems for control purposes, by agencies which often lack the capacity or resources to respond with data-intensive programs using sophisticate suites of chemical analytes. An alternative, is the use of screening techniques that quickly isolate the nature and source of toxicity. In this example, we apply bioassay-directed chemical analyses to water and suspended sediment in a 650 km reach of a major river in Mexico. In addition to agricultural runoff, the river receives many municipal and a wide variety of industrial effluents. Our results successfully demonstrate how this diagnostic approach quickly identifies priorities for monitoring and regulatory attention.

INTERACTION BETWEEN ENVIRONMENTAL RESEARCH AND REGULATION

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Underwood (1995; *Ecological Applications* 5:232-247) discussed four types of research related to environmental management and regulation. Research 1 is the existing research base available to managers for decision-making. Researchers often do not conduct the type of research which would be useful to managers. Managers often request inappropriate research or information from researchers (i.e., ask a stupid question; get a stupid answer). Research 2 is research on the effectiveness of managerial decisions, and is the focus of this presentation. Managers and the public often undervalue scientific information because of the uncertainties associated with that information. Scientific uncertainties are a function of the scientific approach (experimentation; hypothesis-testing; peer review; citation of the literature). If managers and stakeholders adhered to that same approach, they might appreciate that many managerial actions and decisions are also subject to uncertainty. Every managerial decision is a hypothesis or experiment (i.e., research); experimental management is common, or at least commonly discussed, in fisheries science, but not in environmental science. Tests of managerial hypotheses are relatively easy to conduct, and examples will be provided. Managers and bureaucrats traditionally resist evaluation of decision- and policy-making, so scientists must take the lead. Finally, Research 3 is new environmental research conducted to address failed managerial decisions; Research 4 is general research on management. Research 3 cannot be conducted unless Research 2 is conducted to evaluate managerial decisions; Research 4 is the generalized form of the more specific Research 2.

STATUS OF SEDIMENT QUALITY GUIDELINE DEVELOPMENT IN CANADA

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Under the Canadian Environmental Protection Act, the protection of Canada's aquatic and terrestrial ecosystem requires that national environmental quality guidelines be developed for priority chemical substances (CEPA, 1988). Sediment Quality Guidelines are one type of environmental quality guideline presently being developed in Canada for numerous organic and inorganic substances present in sediments. These biological effects-based guidelines are intended to protect aquatic life that are associated with bed sediments in freshwater sediments and marine and estuarine sediments within Canadian waters. This paper provides a brief overview of the principles and processes behind the development of Canadian Sediment Quality Guidelines and presents the current status of their development.

Sediment Quality guidelines are numerical concentrations or narrative statements recommended to support and maintain aquatic life associated with bed sediments and are developed from the available scientific information on the biological effects of sediment-associated chemicals. These guidelines are developed under the auspices of the Water Quality Guidelines Task Group of the Canadian Council of Ministers of the Environment (CCME). The Guidelines and Standards Division of Environment Canada is the technical secretariat for the task group. Sediment Quality Guidelines are intended to be nationally applicable, unlike Sediment Quality objectives, which would be developed in consideration of site-specific scientific (e.g., particle size, acid volatile sulphides, and organic carbon content) or management (e.g., technology, remediation) factors.

As for other Canadian environmental quality guidelines, the goal of Sediment Quality Guidelines is that adverse effects should not be observed for organisms living in, or on, sediments during an indefinite period of exposure to chemicals within the sediments. Therefore, the derivation procedures for Canadian environmental quality guidelines (including water, sediment, soil, and tissue quality) were developed to provide broadly protective tools that will support the functioning of healthy ecosystems (Gaudet et al., 1995; Smith et al., 1996a,b).

Canadian Sediment Quality Guidelines help to evaluate the toxicological significance of sediment-associated substances on aquatic life. These guidelines are being developed for numerous substances (Table 1) following methods described in a formal protocol (CCME, 1995). Under the protocol, there are two methods for deriving sediment quality guidelines: the Spiked-Sediment Toxicity Test approach and the National Status and Trends Program (NSTP) approach, with modifications. These two approaches are outlined in detail elsewhere (CCME, 1995). Together, the two approaches to sediment Quality Guideline development provide complementary information to support the development of national Sediment Quality Guidelines (MacDonald et al., 1992; CCME 1995). The modified NSTP approach, based on Long and Morgan (1990), relies on field data that demonstrate associations between chemical concentrations in sediments and biological effects in organisms exposed to those chemicals in sediments, whereas the SSTT approach establishes cause-effect relationships between individual chemicals or specific mixtures of chemicals spiked in sediments and organisms exposed under controlled laboratory conditions.

Table 1. Substances for which Canadian Sediment Quality Guidelines are being derived.

Trace Metals	Polycyclic Aromatic Hydrocarbons (PAHs)[2]	Organochlorines
arsenic[2]	acenaphthene	bis(2-ethylhexyl)phthalate
cadmium[1]	acenaphthylene	chlordane[2]
chromium[2]	anthracene	*p,p'*-DDD[2]
copper[2]	fluorene	*p,p'*-DDE[2]
lead[2]	2-methylnaphthalene	*p,p'*-DDT[2]
mercury[1]	naphthalene	DDT, total[2]
nickel	phenanthrene	dieldrin[2]
silver	benz(a)anthracene	dioxins and furans[2]
zinc[2]	benzo(a)pyrene	endrin[2]
	chrysene	heptachlor epoxide[2]
	dibenz(a,h)anthracene	lindane[2]
	fluoranthene	total PCBs[2]
	pyrene	Aroclor 1254[2]
		toxaphene[2]

[1] Interim sediment quality guidelines approved by CCME. Technical supporting document available.
[2] Technical supporting document in preparation.

Using the NSTP approach, two concentrations of the substance are recommended. The lower of these concentrations, the threshold effect level (TEL), is the concentration below which adverse biological effects would be expected to occur rarely. The higher of these two concentrations, the probable effect level (PEL), is the concentration above which adverse biological effects would be expected to occur frequently (i.e., ≥ 50% of the time) (CCME, 1995). Sufficient spiked-sediment toxicity data to derive Sediment Quality Guidelines using the SSTT approach are currently available for only a few substances, such as Cd and Cu. Although information exists to support the development of SSTT values for a few substances, methodological considerations, concerns regarding spiked-sediment toxicity testing methodology (Environment Canada, 1995) limit the degree to which these values may be used as the scientific basis for recommending full Sediment Quality Guidelines at this time. Therefore, the TELs calculated using the modified NSTP approach are most likely to be adopted as interim Sediment Quality Guidelines. The PELs provide information, in addition to interim Sediment Quality Guidelines, regarding the potential for observing adverse biological effects at higher concentrations. The guideline development process also includes a review of additional supporting scientific information which is compiled into a technical document along with the guideline numbers.

Interim Sediment Qality Guidelines and PELs for freshwater and marine sediments have been derived for a number of chemicals (Table 1). Canadian Sediment Quality Guidelines have been approved and recommended by the CCME for Cd and Hg in 1996 and 1997, respectively. Sediment Quality Guidelines for several other metals (e.g., As, Cr, Cu and Zn) and organic compounds (e.g., polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and other organochlorines) are currently undergoing national review. The draft interim Sediment Quality Guideline numbers have been published in conjunction with the proceedings of a previous conference (Smith et al., 1996a). Detailed chemical-specific supporting technical documents for several of these substances are currently in preparation. CCME approval of the new guidelines is expected to be granted in early 1998 (Table 1).

Canadian Sediment Quality Guidelines and PELs are flexible interpretive tools for evaluating the toxicological significance of sediment chemistry data, as well as for prioritizing actions and management decisions. Sediment chemical concentrations below the Sediment Quality Guidelines are not expected to be associated with adverse biological effects, while concentrations above the PELs are expected to be frequently associated with adverse biological effects. Chemical concentrations between the Sediment Quality Guidelines and PELs represent the range in which effects are occasionally observed. The use of these two values is a practical means of characterizing sites as being of minimal, potential, or significant toxicological concern in order to focus further investigations.

As our understanding of the impacts of toxic substances on aquatic organisms continues to evolve, refinements to the guideline development process may be required. We recommend that Sediment Quality Guidelines be used in conjunction with additional sediment quality assessment tools (e.g., consideration of background concentrations, other assessment values such as PELs, biological assessments, and additional environmental quality guidelines for other media). Additional sediment quality guidelines will be derived for new substances as sufficient data required by the protocol (CCME, 1995) becomes available. The simultaneous use of multiple Sediment Quality Guidelines provides an effective tool for assessing, maintaining, protecting, and improving sediment quality in Canada.

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STATUS OF SOIL QUALITY GUIDELINE DEVELOPMENT IN CANADA

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The Canadian Council of Ministers of the Environment (CCME) recently published soil quality guidelines for 20 substances. These National Soil Quality Guidelines are derived using current toxicological data, according to the formal protocol established in 1996, and are intended to be protective of ecological and human health. They provide the scientific basis for the assessment and remediation of soil for specific land uses (agricultural, residential/parkland, commercial and industrial) and generic exposure scenarios. Soil quality guidelines consider key receptors, exposure pathways, and the protection of specific resource uses. These achievements represent one of the first set of risk-based, soil quality guidelines for protecting environmental and human health in the world. In this paper, we will present the current status of soil quality guideline development in Canada, provide an overview of the principles and processes behind soil quality guideline development for ecological receptors, and discuss the decision-making context within which the guidelines are being used. The application of soil quality guidelines to the remediation of contaminated sites will be illustrated. We will also discuss some of the key issues facing solid quality guideline development in the future.

A FRAMEWORK FOR ENVIRONMENTAL MANAGEMENT IN CANADA: FROM GENERIC ENVIRONMENTAL QUALITY GUIDELINES TO SITE-SPECIFIC ECOLOGICAL RISK ASSESSMENT

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The maintenance, protection and restoration of a high level of environmental quality requires the availability of practical scientific tools. The Canadian environmental management framework incorporates the use of several such tools, including national environmental quality guidelines (for the protection of uses of water, sediment, biota, and soil), site-specific objectives, and site-specific Ecological Risk Assessment (ERA). The effective use of these tools lends consistency and transparency to potentially complex decision-making processes that must balance social, economic, and environmental factors. Environmental quality guidelines define levels of substances in environmental media which, if not exceeded, will protect the environment for specified use(s) of land and water. These guidelines have a number of important uses, including identifying sites or toxic substances of concern, assessing the significance (in terms of potential risk posed) of contamination, and determining the need for further assessment or action. Guidelines also serve as the scientific basis for the development of site-specific objectives. These objectives incorporate information that is available for a particular site, such as data on contaminant mobility and local receptors. In cases where further assessment is warranted, ERA may be appropriate. Such cases may occur when, for example, national guidelines do not exist for a toxic substance of concern, clean-up to guideline levels is not feasible for the targeted uses of the site, guideline-based objectives do not seem appropriate given the site-specific conditions, particularly significant or sensitive receptors have been identified, or there are issues of significant socio-economic concern. The Canadian ERA framework provides a consistent basis for understanding, implementing and communicating ecological risk assessments. It includes guidance on deciding when an ERA should be conducted, and describes key steps and considerations in planning and implementing an ERA. Environmental quality guidelines are an important part of this framework, as the initial use of guidelines focuses investigations on the areas and substances of greatest concern. This presentation will discuss the essential features of each of the above tools, using Cu as an example, and will illustrate how the Canadian environmental management framework integrates their use into an effective decision-making process.

AN OVERVIEW OF THE FRAMEWORK FOR CONDUCTING ENVIRONMENTAL ASSESSMENTS OF PRIORITY SUBSTANCES UNDER THE CANADIAN ENVIRONMENTAL PROTECTION ACT

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This paper presents an overview of the framework for conducting environmental assessments of priority substances under the Canadian Environmental Protection Act, with emphasis on the tiered approach for risk characterization. Risk characterization compares exposure of a substance in various environmental compartments with laboratory or field effects data in order to determine if the substance is likely to cause adverse environmental effects. A tiered approach is used to ensure that assessments proceed only to the level of refinement required for effective decision-making. Tier 1 involves hyperconservative point estimates of exposure and effects. Tier 2 involves more realistic, but still conservative, point estimates of exposure and effects. Tier 3 involves comparison of distributions of exposure and effects. A case study of a risk characterization of a priority substance, butylbenzylphthalate, will be presented.

ASSESSMENT OF TAINT IN FISH: THE SCIENCE BEHIND THE FLAVOUR

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Taint in fish flesh is defined as the abnormal odour and/or flavour which occurs in the edible tissue compared to a control. Tainting may result from either postmortem spoilage or from flavour compounds introduced into the fish from the water or uptake from the dietary food chain or via a contaminated environment. Taints occur naturally and from anthropogenic sources. In many instances, the presence of 'tainting' compounds may not have a detrimental effect on the health and condition of the fish nor do they pose a human health risk. However, their presence at a level greater than or equal to an individual's smell and taste threshold can lead to rejection of the food as tainted and unacceptable for human consumption. This can have wide ranging socioeconomic and legal implications for groups dependent on fishing for their livelihood and industry (uncontrolled releases and regulated discharges). Traditionally, sensory assessment to detect 'tainting' has been treated more as an art than as a science. The literature on the study of tainted fish is confounded by the incorrect selection and/or use of sensory methodology and panelists. The objective of this presentation is to address issues and concerns about conducting standardized sensory evaluations on tainted fish in terms of: [1] exposure and handling fish for testing; [2] selection, screening and training of the analytical sensory panel members; [3] quality assurance and control practices; and [4] data analyses and interpretation of the results. Real data and case studies will be used to illustrate key points of the presentation.

THE LIMITATIONS OF AQUATIC TOXICOLOGY IN ENVIRONMENTAL ENFORCEMENT: CAN ONE DEAD *Daphnia* PUT A PERSON IN JAIL?

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Governments are increasingly relying upon aquatic toxicological studies and standards ostensibly based on studies in prosecutions of individuals and companies under provincial and federal environmental laws. Convictions under these laws require "proof beyond a reasonable doubt" but often toxicology cannot offer such a high level of proof. This presentation will suggest that the limitations of aquatic toxicology sit uneasily with the requirement of proof beyond a reasonable doubt in our legal system. It will also offer comments concerning the proper role of aquatic toxicological studies and standards in environmental enforcement.

POSTER PRESENTATIONS/SÉANCES AFFICHES

in Vitro Methods

THE EFFECT OF CORTISOL AND NONYLPHENOL ON GROWTH AND ORNITHINE DECARBOXYLASE ACTIVITY OF JUVENILE ATLANTIC SALMON, *Salmo salar*

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INTRODUCTION

Sublethal concentrations of chemicals which lead to chronic conditions of ill health may result in a small but continuous reduction in population. Stresses inflicted on an organism's mechanisms for maintaining a healthy physiological state may cause changes in histological properties and behavioral, physiological and biochemical processes. This study is part of a project to develop such responses into a series of diagnostic tests and techniques to identify conditions of ill health in aquatic animals, communities and populations.

Laboratory studies have indicated that stress in fish caused by chronic exposure to chemicals or unfavorable environmental conditions results in decreased growth rates. However, it is not possible to determine unequivocally decreased growth in fish populations from field samples. One potential biochemical measure of short-term growth rates in fish is the activity of the enzyme, ornithine decarboxylase. It is the first and rate-limiting enzyme in the biosynthesis of polyamines which are essential for the biosynthesis of macromolecules such as DNA, RNA and proteins. Increases in ornithine decarboxylase activity should precede other biochemical changes commonly used as indices of instantaneous growth in fish. This laboratory study evaluates the potential of ornithine decarboxylase activity as an indicator of growth rate and health of juvenile Atlantic salmon.

METHODS

Juvenile Atlantic salmon, *Salmo salar* (5 to 15 g, 7 to 11 cm), were anesthetized with 1% 2-methyl-2-butanol and a pit tag was implanted into the anterior intestinal cavity. The salmon were held in flow-through fresh water at ambient temperature (2.5 to 7°C), under natural photoperiod and fed daily, except on treatment and sampling days, throughout the experiment. After 10 d (day zero of experiment) the fish were anesthetized, and their weight and length measured. Salmon, 40 per group, were administered intraperitoneally 200 mg/kg of nonylphenol in vegetable oil, 50 mg/kg of cortisol in vegetable oil:shortening (1:1), or vegetable oil:shortening (1:1, sham group). Ten salmon from each treated group and controls were placed in each of four tanks containing 60 L of fresh water. All fish were anesthetized and retreated on days 14, 35, 56 and 77. All the salmon from one of the four tanks were sampled on days 20, 41, 62 and 83.

The salmon were anesthetized by adding MS-222, 100 mg/L prior to sampling. The salmon were killed with a blow to the head, the tail severed and blood from the caudal vein was collected in hepranized tubes. The blood was centrifuged (5500 x G), the hematocrit determined and the plasma collected and stored at -95°C. The liver and a section of the left dorsal anterior expaxial muscle were dissected, placed on ice, then homogenized in sucrose (250 mM) and Tris HCl (10 mM) pH 7.6 buffer. The homogenates were centrifuged (20,000 x G, 30 min, 5°C) and aliquots of the supernatant were stored at -95°C until analyzed for protein concentration and ornithine decarboxylase activity.

Plasma cortisol was determined by radioimmunoassay (Reddy et al., 1995) with a radioimmunoassay kit (Incstar Corp.) and a LKB-Wallac Model 1272 gamma counter. Ornithine decarboxylase activity was determined by an enzymatic procedure (Benfey, 1992) optimized for salmon liver. L-^{14}C-Ornithine is used as substrate and the liberated ^{14}C-carbon dioxide is captured and measured with a LKB scintillation counter.

Percent change in weight and length were determined by comparing measurements for each individual at day zero to that measured on the sampling day. The findings are preliminary and statistical analyses, protein and ornithine decarboxylase assays are incomplete.

RESULTS AND DISCUSSION

The plasma cortisol concentrations and hematocrit in the control, and all treatment groups were indicative of stressed salmon. Hematocrit in all groups ranged from 41 to 60%. The normal hematocrit for juvenile Atlantic salmon is near 31%. Plasma cortisol concentrations for the controls, sham and nonylphenol groups ranged from 17 to 59 µg/kg. In previous studies, normal unstressed resting plasma cortisol concentrations for this stock of salmon were less than 20 µg/kg. The most likely cause of the stress to these fish is their response to MS222 and the physical stress during sampling.

There was no significant difference in the plasma cortisol concentrations between controls, sham and nonylphenol treated groups, nor between day 0 and day 83 concentrations within each of these three treatment groups. The plasma cortisol concentrations in the cortisol treatment group (range of means was 810-4313 µg/kg) were greater than the maximum (450 µg/kg) that can be achieved by adaptation mechanisms in salmonids.

After 83 d the control group, sham, and nonylphenol treated salmon had significantly increased in weight from day 0 by 59, 31 and 32% and in length by 12, 6 and 6%, respectively. The cortisol treated group had no significant growth after 83 d. A similar order was observed for liver ornithine decarboxylase activities, which were controls > sham = nonylphenol > cortisol treated salmon (3329, 2186, 2040 and 1194 mmole ornithine/h/g wet wt, respectively).

These results are preliminary and some enzymatic and statistical analyses are incomplete. However, the results indicate that high concentrations of plasma cortisol inhibits growth in salmon. The effect of nonylphenol on growth is not definitive as the growth of salmon in this treatment group was not different from the sham group. The results also suggest that liver ornithine decarboxylase activity may be indicative of instantaneous growth rate.

This study suggests that liver ornithine decarboxylase activity may be an indicator of health of fish from a single sampling in the field if it is assumed that healthy unstressed fish utilize energy for active growth. Further research is required to determine the normal range of ornithine decarboxylase activity in relation to life stage and size.

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THYROID FUNCTION IN LAKE TROUT (*Salvelinus namaycush*) EXPOSED TO CO-PLANAR 3,3',4,4',5-PENTACHLOROBIPHENYL

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Recent studies indicate that co-planar PCB congeners or their metabolites can distort thyroid function in mammals. Although co-planar PCBs have been detected at μg/kg levels in fish from contaminated areas, few studies have examined the potential of co-planar PCBs to alter thyroid function in fish. We treated immature lake trout by dietary gavage with vehicle containing 1.2 or 40 μg 3,3',4,4',5-pentachlorobiphenyl (PCB 126)/kg fish weight. Blood and tissue samples were collected after 8 wk. The treatments produced sustained dose-dependent elevations of tissue PCB 126 concentrations. Thyroid epithelial cell height (TECH), plasma thyroxine (T_4) and 3,3',5-triiodo-L-thyronine (T_3) concentrations, hepatic 5'-monodeiodinase, hepatic glucuronidation of T_4 and T_3, as well as plasma T_4 kinetics were analyzed. Exposure to PCB 126 caused changes in TECH, plasma T_4 kinetics and T_4-glucuronidation. Despite these changes, plasma thyroid hormone concentrations, 5'-monodeiodinase and T_3-glucuronidation were unaltered by PCB 126 exposure. No effects on fish growth or condition were observed.

IMMUNOTOXICOLOGICAL STUDIES IN THE HARBOUR SEAL (*Phoca vitulina*)

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A number of unrelated virus-induced mass mortalities among marine mammals during recent years has raised concerns about the possible contribution of immunotoxic chemicals. Many of the populations affected by these epizootics inhabited industrial coastal areas of Europe and North America, and had high concentrations of immunotoxic polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) in their fat reserves. Following the *Morbillivirus*-related mass mortality of 20,000 harbour seals (*Phoca vitulina*) in Europe in 1988, we carried out a study in which 22 juvenile seals were fed herring from either the contaminated Baltic Sea or from the relatively uncontaminated Atlantic Ocean. During the 30 month study, seals of the Baltic group had lower *in vitro* natural killer (NK) cell activity, and lower *in vitro* and *in vivo* T-cell function, than seals of the Atlantic group. The Baltic seals had accumulated 209 ng/kg of 2,3,7,8-TCDD toxic equivalents (TEQ; lipid weight) in their blubber, compared to 62 ng/kg TEQ in the Atlantic group. PCBs contributed approximately 95% to the total TEQ value in seal blubber, and therefore presented the greatest 'dioxin-like' risk from the PCBs, dioxins and furans to the seals. We conclude that environmental contaminants, notably PCBs, may have contributed to the severity and extent of recent virus-associated mass mortalities among marine mammals. Given the stable chemical characteristics of such contaminants and their continued entry and cycling in the environment, animals occupying high trophic levels may continue to suffer from adverse biological effects well into the 21st century.

FLUORESCENCE QUENCHING OF PAHs: POTENTIAL EXPERIMENTAL ARTIFACTS DURING THE DETERMINATION OF K_{doc} VALUES

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Fluorescent quenching has been a popular method for measuring the binding constants for a variety of PAHs with dissolved organic matter in surface and groundwater. An investigation was made using benzo(a)pyrene, benz(a)anthracene, pyrene, fluorene, phenanthrene, and anthracene and humic matter from three aquatic sources. The humic material was concentrated by reverse osmosis before being used. The factors which will be discussed are cuvette wall sorption and desorption, photodegradation, and oxygen quench. Cuvette wall sorption/desorption is related to the K_{ow} of the PAH. Photodegradation of the PAH is dependent on the choice of instrument slit widths and selected wavelengths. Oxygen quench of the PAH fluorescence occurs with all PAHs. However, trying to adjust conditions to eliminate oxygen causes more problems than it solves. An equilibrium technique will be presented which yields reproducible data. The K_{doc} values for the investigated PAHs range from a low of 2.6×10^4 ml/g for phenanthrene and anthracene to 2.3×10^5 ml/g for benzo(a)pyrene. One of the DOC sources was fractionated (ultrafiltration) according to molecular size and the majority of the binding activity was found in the higher molecular weight fraction (>10K MWCO).

ARCELLACEANS: SHELLED MICROINVERTEBRATES AS COST-EFFECTIVE TOOLS FOR MONITORING CURRENT AND HISTORICAL ENVIRONMENTAL TRENDS

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Arcellaceans (thecamoebians) are freshwater microscopic protozoans that form agglutinated shells. They occur in lacustrine sediments and have been used to reconstruct Pleistocene-Holocene paleoenvironments. Research has also demonstrated the use of arcellaceans as excellent indicators of the environmental effects of industrial activity. As examples, we have been able to demonstrate a relationship between the distribution of arcellacean faunal assemblages and arsenic and heavy metal contamination in mine tailings in lakes near Cobalt, Ontario. Similar studies of cores from Italian acidic lakes polluted with copper and ammonium sulfate have documented the long-term history of pollution and recovery. There are few benthic environmental indicators in lacustrine environments with the potential for such broad utility as arcellaceans. Like macro- benthic invertebrates, some arcellacean species have been shown to be more sensitive to pollution than others. In addition, research has documented within-species 'morphing' in response to environmental stresses. As they reproduce rapidly (generation times of a few days) arcellaceans can provide a short term indicator of ecosystem health. Since their shells preserve extremely well they may also be used to assess long-term environmental trends. Arcellaceans are extremely abundant, with tens of thousands of specimens occurring in a single teaspoon, making them ideal for statistical analysis. Sample collection for arcellaceans is similar to that for macro- benthic invertebrates. Sample preparation is simplified, as no preservative is required. In the laboratory, specimens are sorted under a microscope, and after about a month of training, a technician can sort a typical sample in about one hour.

YOU CAN'T EAT WHAT YOU CAN'T SEE: A TALE OF FISH STARVATION

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Simulated effluent from Iron Ore Company of Canada was tested to evaluate the incorporation of a floatation process utilizing an amine based reagent to reduce silica content of iron ore. Much of the assessment focus was on the use of the amine reagent and its acute and sublethal toxicity. When fathead minnow sublethal tests were conducted on the amine containing effluent, growth was impaired. However, the effluent was also a dark orange colour and generally opaque from iron oxide formation. Parallel tests were conducted without amine in the effluent and the results were identical as with the amine. The exposure was repeated and just as mortality began fry were sacrificed for histopathological analysis. Examination of fry showed that gills were clear (no respiratory impairment) and the guts of exposed fry were empty while controls were full of food. The fry exposure tests included daily feeding. It was concluded that mortality resulted from starvation because of an inability to see and secure food. The impact assessment could then be based on iron content and light transmission as it was clear that the amine reagent had no effect at the concentrations used.

THE EVALUATION OF MICROSCALE BIOASSAYS AS TOXICITY MONITORING AND IDENTIFICATION TOOLS FOR PULP AND PAPER EFFLUENT

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The use of four micro-scale assays for toxicity monitoring and identification was investigated to evaluate their performance and usefulness compared to conventional assays (i.e. *Daphnia magna*, rainbow trout and fathead minnow). The first objective of this study was to identify which micro-scale assays were most representative of regulatory tests' response. These assays could then be used as a screening tool to monitor effluent toxicity and to evaluate the potential toxicity of chemical products used in mill. The use of the micro-scale assays would represent a major advantage as it would allow the mill personnel to conduct the tests in house and obtain results rapidly in order to be able to react more quickly to toxicity events. The second objective was to evaluate their performance and usefulness in Toxicity Identification Evaluation (TIE) programs for Pulp and Paper effluents (following well known U.S. EPA procedure). During the preliminary evaluation, the micro-scale assays (Daphtoxkit^{TM}, Daphnia I.Q.^{TM}, Thamnotoxkit F^{TM} et Microtox^{TM}) were evaluated and compared to the standard *D. magna* assay using effluent samples collected from five different types of mill operation. Based on the test results, two micro-scale assays were retained to pursue the evaluation and one type of effluent was selected in order to perform the TIE. The Phase I TIE was performed with the two selected micro-scale assays in addition to the conventional tests (*D. magna* and fathead minnow). The micro-scale assays provided similar results to those of the conventional assays, indicating that they could prove useful for screening and monitoring toxicity.

THE USE OF CHEMICAL TRACERS TO MONITOR THE EXPOSURE OF INDIGENOUS FISH POPULATIONS TO PULP MILL EFFLUENTS - TRACER WORKING GROUP

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Please see page 64 for the abstract.

EEM TRANSITION TO CYCLE 2, INFORMATION MANAGEMENT DEMONSTRATION

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This demonstration allows hands on viewing and trial of the existing Cycle 1 Information Management Electronic Reporting System and accompanying instructions. The demonstration will be accompanied by an opportunity to discuss potential changes to this format, in order to accommodate Cycle 2 information. The changes to the Cycle 1 reporting format will also address some omissions noted during the use and implementation of the Cycle 1 Electronic Reporting System. In addition, a simple geographical query application, intended to demonstrate one of the many potential ways users may wish to view or access this data will be available for demonstration. The structure of the EEM receiving database will be available for examination by interested parties as well.

LIVESTOCK MANURE POLLUTION PREVENTION PROJECT

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Environment Canada has initiated a pollution prevention project to tackle the issues surrounding manure handling and application that have lead to manure spills and related fish kills and habitat degradation across Ontario. The working group for this Livestock Manure Pollution Prevention Project, sponsored by Environment Canada, includes representatives from the agricultural industry, academic institutions and government agencies. This proactive step comes at a time when the province is experiencing a period of intensification in the livestock industry, particularly housing facilities. Manure management is a complex issue. Many individuals, organizations and agencies are presently researching and demonstrating innovative technology so that the increased amount of manure will be handled and applied without negatively impacting the environment. The Working Group provides a forum to encourage practical ideas about manure management, promote research and encourage Ontario farmers to adopt best management practices that will help reduce manure spills and resulting environmental impacts.

Wastewater

CONSTRUCTED WETLANDS: SINKS OR SOURCES OF TRACE METALS?

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The capacity of a constructed wetland (CW) to retain trace metals was examined in relation to changes in water detention time and other variables. Hydrological measurements and duplicate water samples were taken every 3 d from April 24 to August 19, 1997 at the Monahan CW in Kanata, ON. This 32700 m^2 CW is one of the largest one in Canada. It was built in 1995 to receive the runoff from agricultural and residential watershed of 637 hectares. The CW was an overall sink for Fe, Mn, Zn, Cu and U but was a net source of As. Nominal water detention time had an impact on Fe and Zn retention only and a minimum water detention time of 5 d was necessary for 70% retention. Longer detention times (>15 d) resulted in less Fe removal while Zn retention reached a maximum after 10 d. Time of the year (spring vs summer) had important impacts on retention of some metals. As production and Zn retention reached a maximum in late summer while Fe retention reached a peak in late May followed by a decrease and a higher variability in mid-late summer. Biological respiration is likely to play a major role in the retention processes. By lowering the redox potential of the sediments, respiration leads to metal remobilization. Under these conditions, CWs tend to be sources rather than sinks for metals. This situation is likely to be true for CW receiving nutrient rich runoff. This work will be published later on this winter under the same authors and same title.

CHRONIC TOXICITY OF HIGH AND LOW DENSITY UV DISINFECTED EFFLUENTS FROM SELECTED SEWAGE TREATMENT PLANTS IN ONTARIO

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UV irradiation is currently used in disinfection of treated wastewater effluents as a viable alternative to chlorination, which may lead to chronic toxicity effects of residual chlorine in the aquatic environment. Some current literature supports the hypothesis that UV light can induce changes in certain organic compounds, which become more toxic after UV irradiation. Concern was expressed by the Ontario Ministry of Environment and Energy (MOEE) about the potential of UV disinfection to induce toxicity in municipal sewage treatment plant effluents. Samples of non-disinfected effluent, UV disinfected effluent and chlorinated/de-chlorinated effluent were tested for toxicity at three different sewage treatment plants in Ontario. One plant used low intensity UV and another used high intensity UV light for disinfection. The third plant did not have UV disinfection, and therefore bench scale UV irradiation had to be utilized. Two types of 7 d chronic toxicity tests, using the cladoceran (*Ceriodaphnia dubia*) and fathead minnow (*Pimephales promelas*), were performed on these samples. No significant indications of UV-induced toxicity were found in the effluent samples tested, regardless of location or intensity of UV treatment. Some samples did show indications of toxicity after bench scale chlorination/de-chlorination. The majority of samples tested were non-toxic, however effluent samples which were toxic before disinfection were also toxic after disinfection. The chronic toxicity testing performed on the samples studied confirms the results of similar studies: the levels of UV irradiation required for disinfection purposes, do not appear to increase effluent toxicity. In that sense, UV disinfection was found superior to chlorination/de-chlorination which did increase toxicity of some effluent samples and could potentially harm the receiving water ecosystems.

Soil-Sediment Assessment

A SIMPLIFIED ALTERNATIVE TO WATER RENEWAL SYSTEMS FOR CONDUCTING SEDIMENT TOXICITY TESTS WITH FRESHWATER INVERTEBRATES

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Standard protocols for conducting sediment toxicity tests call for water-renewals of one to four volume additions per day of overlying water. The water renewal is recommended in order to maintain water quality characteristics. Static tests tend to show increases in alkalinity, hardness, and conductivity as well as some metabolic products such as ammonia or even decreases in pH due to high sediment sulfide content. A sediment test chamber consisting of a one litre, polycarbonate cone was developed to eliminate the need for water-renewal systems thus simplifying and reducing the cost of sediment toxicity testing. The cone chamber, allowing water:sediment ratios up to 100:1, was assessed for water quality characteristics as well as density effects on survival and growth of *Hyallela azteca* and *Chironomus riparius* in comparison to the standard static beaker test. The increase in overlying water conductivity was greatly reduced in the cones relative to levels found in the standard beaker test. Survival of both *Hyalella* and *Chironomus* were not affected by the cone, but both species demonstrated increased growth in comparison to the standard beaker test. The cones provided constant overlying water conditions and one litre of water for chemical analysis at the end of the experiment. Field sediment samples containing a high sulfide content were also evaluated for their effect on overlying water quality and were subsequently assessed using the cone chamber.

A METHOD FOR AMMONIA REDUCTION IN WHOLE SEDIMENTS

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Ammonia is a contaminant of concern and can confound toxicity associated with contaminated whole sediments. It is therefore beneficial to be able to limit the impact of ammonia on toxicity originating from whole sediment by using methodology which identifies and reduces interstitial ammonia levels. Ammonia values are measured prior to seeding tests to determine background levels of ammonia. A comparison of background values to a target value (species specific), determines if ammonia purging is necessary. Should purging be required, the process begins the day after sediment distribution and applies to all replicates for all treatments including the negative control and any references. The purging process involves two volume renewals per day. The length of the purging period will depend upon the degree of difference between the observed values/ background and the target value. Once all the ammonia values meet or are below the target value, the test can be seeded and proceeds as usual. A concurrent reference toxicant test is also performed to determine the sensitivity of the batch of organisms to ammonia. Results obtained from testing over a period of twelve months consistently show an efficient way of determining interstitial ammonia levels and a reduction in the ammonia values over time during the purging process.

THE INCORPORATION OF MICROSCALE BIOASSAYS INTO A TIERED FRESHWATER SEDIMENT TOXICITY METHODOLOGY

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Effective assessment of sediment toxicity requires cost-effective screening tools. The use of several micro-scale assays for evaluating freshwater sediment toxicity were investigated to incorporate in a representative and cost-effective test battery. The bioassays evaluated (20 assays in total) included micro-scale assays performed on solid phase, pore water and organic extracts as well as conventional standardized sediment assays with *Chironomus riparius* and *Hyalella azteca* on 15 sediment samples collected in the St. Lawrence River/Great Lakes systems. Selected sediment physical and chemical

analyses were also conducted to aid in the interpretation of results. The main objective of this study was to assess the performance and usefulness of various micro-scale bioassays in detecting the toxic potential of freshwater sediments. Several qualitative and quantitative criteria were established for evaluating the performance and usefulness of micro-scale bioassays. A general index of performance was attributed to each bioassay by summing points scored for each criterion. The use of micro-scale assays in a strategy for evaluating sediment contamination could prove very useful for screening and monitoring toxicity. By targeting regions where sediment contamination poses a risk for both benthic organisms and organisms living in the water column, these assays could serve to optimize sediment and water sampling programs at sites where dredging or remediation are planned.

BURROWING MAYFLY (*HEXAGENIA*) POPULATIONS IN LAKE ERIE

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Hexagenia mayflies, which formerly dominated the soft sediments of Lake Erie and other Great Lakes mesotrophic habitats, were eradicated in the 1950s. In western Lake Erie, adult *Hexagenia* were observed at isolated locations in 1991 following 20 years of reduced phosphorus inputs and the invasion of zebra mussels. Semi-annual benthic surveys have documented expansion of the range of *Hexagenia* larvae from west to east, and two- to four-fold annual increases in density. In 1997, larvae from high-density sites (up to 1,500 m^{-2}) exhibited distinct size bimodality, suggesting that density-dependence may ultimately limit growth and impose a two-year life cycle. Adult *Hexagenia* have been observed throughout western Lake Erie but in only isolated shoreline locations in central or eastern basins. The continued absence of larvae north of Pelee Island suggests that benthic conditions rather than lack of colonists may be limiting recovery in some regions. Body burdens of organic contaminants in adult mayflies are greatest at western-most and midbasin locations of western Lake Erie.

THE USE OF A NEW SPRINGTAIL SPECIES (*Onychiurus folsomi*) IN A REPRODUCTIVE TEST WITH CONTAMINATED SOILS

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A terrestrial reproductive test using a new species of springtail (*Onychiurus folsomi*) was developed for assessing the toxicity of contaminated soils from Western Canada. Ten adults were placed into each test unit which consisted of a 120 ml glass jar with 30 g of test soil (w.w) at 25-35% moisture by weight depending on the individual water holding capacity of each treatment. The two control soils used as site-soil diluents included a formulated artificial soil (AS) and a reference soil (RS) collected from a clean site close to where the contaminated soils were obtained. The test duration of the definitive test was 35 d and the measurement endpoints were adult mortality, fecundity, and total number of young produced in each treatment. Pre-screening acute toxicity tests generated adult 7 d LC_{50}s of 8.1 and 10.1% condensate-contaminated soil (d.w.) and 3.0 and 9.9% amine-contaminated soil (d.w) for the site soils diluted with AS and RS, respectively. In the definitive reproduction tests the 35 d LC_{50}s were 16.9 and 21.6% condensate-contaminated soil (d.w) diluted with AS and RS, respectively, which suggests that there is significant variation in either the toxicity of different batches of the condensate site soils or differences in sensitivity of the test organisms. The former is more likely given that the 35 d LC_{50}'s were 1.5 and 2.5% for the amine-contaminated soil (d.w.) diluted with AS and RS, respectively. Fecundity was lower in tests where organisms were exposed to amine-contaminated soil compared to those exposed to soils contaminated with condensate. Fecundity was a more sensitive toxicity endpoint than adult survival in soils contaminated with amines but not condensate contaminants.

Petroleum/PAHS

THE EFFECTS OF OIL SANDS MINE TAILINGS ON THE DIET OF YELLOW PERCH (*Perca flavescens*)

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Oil sands are mined in northern Alberta for bitumen which is then converted to synthetic crude oil. A land reclamation strategy (the 'wet landscape' option) has been developed in which mature fine mine tailings (MFT) are placed in the bottom of a mined out pit and then capped with approximately 4-6 m of clean water. The diet of a population of yellow perch stocked in such a constructed lake was monitored throughout the summers of 1995, 1996 and 1997, and compared with the stomach contents of yellow perch from reference lakes in the area. Relative importance (abundance and frequency of occurrence) of prey in the diet was substantially different between lakes: yellow perch in the constructed lake ate predominantly cyprinids (60% of prey consumed), gastropods (20%) and amphipods (15%), while stomachs of yellow perch in the reference lakes contained almost exclusively aquatic insects, mainly Diptera. Benthic invertebrate samples collected from each of the lakes suggest that the diets of the yellow perch populations reflected food availability, and that the oil sands mine tailings had an indirect effect on the quality of the yellow perch habitat.

AN ECOPHYSIOLOGICAL ASSESSMENT OF THE TOXICITY OF OIL SANDS TAILINGS TO FRESHWATER LEECHES

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Wet landscape reclamation is one option for reclamation of oil sands tailings. The tails are capped with water to form lakes. This study examined the toxicity of oil sands tailings to the freshwater leech *Nephelopsis obscura* in simulated wet landscape microcosms. Leeches are a vital component of freshwater ecosystems living in and on the sediments and in the water column. They feed on invertebrates and serve as a food source for fish. The objective of this investigation was to use a bioenergetic model for energy acquisition and allocation to assess environmental quality. Three size classes of leeches were exposed for 12 wk to either a control sediment, tailings capped with control sediment, and tailings. Each sediment was covered with 3 m^3 of uncontaminated pond water. The parameters assessed included survival, growth, ingestion, faeces-plus-mucus production, ammonia production, resting and active respiration, lipid levels and reproductive maturity. The uncapped tailings were acutely lethal to all size classes of leeches. Leeches in the capped tailings treatment survived and there were no measured effects on ingestion, faeces-plus-mucus production, absorption efficiency, resting and active respiration, aerobic scope and lipid levels. However, compared to controls, the leeches in the low treatment had reduced ammonia production, reflecting greater efficiency in the assimilation of food. Although exposure to the low treatment did not affect reproductive maturity, these

leeches produced fewer cocoons and hatchlings. These findings are discussed in light of the wet landscaping reclamation plan and the utility of a bioenergetic approach to assessment of environmental quality with leeches and other organisms.

PRELIMINARY RESULTS OF A CAGED BIVALVE STUDY AT 70 METERS IN PORT VALDEZ, ALASKA

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An *in-situ* field study was conducted to evaluate the feasibility and scientific value of using caged mussels as a monitoring tool to characterize chemical exposure and biological effects associated with the Ballast Water Treatment Facility (BWTF) effluent being discharged into Port Valdez, Alaska. A tiered approach was used; Tier 1 assessed mussel survival and growth; in Tier 2 mussel tissues will be analyzed for chemicals of concern. Caged mussels (*Mytilus trossulus*) were transplanted at seven sites in the vicinity of the BWTF diffuser at a depth near 70 m. Three hundred mussels were deployed for 56 d at each station; cages with individual compartments were used to facilitate measurement of individuals at the beginning and end of the test. There were no statistically significant differences in either weights or lengths by station at the beginning of the test. Mean survival of mussels was 97% and there were small increases in mean length (5%) and weight (7%). Growth results were similar to other mussel transplants in southeast Alaska and natural populations of intertidal mussels in the vicinity of the BWTF. The Tier 1 criteria were met and the pilot study demonstrated that mussels transplanted to depths near 70 m in Port Valdez will survive and grow. The growth endpoint served several different functions: [1] A criterion for test acceptance and advancement to the next tier of testing; [2] An effects endpoint to evaluate organismal response; and [3] A method for calibrating bioaccumulation with changes in tissue weight.

SEDIMENTATION OF HYDROCARBONS IN THE CORNWALL-LAKE ST. FRANCIS REACH OF THE ST. LAWRENCE RIVER

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A Long Term Sensing Site network was established in the Cornwall-Lake St. Francis reach of the St. Lawrence River to aid in documenting the rivers response to remedial measures being implemented in the Cornwall-Massena reach of the St. Lawrence River. The known primary contaminants of concern are polychlorinated biphenyls (PCBs). However, the toxicology of chemical mixtures, not single chemicals, is a real issue regarding effects of environmental exposure. Therefore, the distribution of selected polycyclic aromatic hydrocarbons (PAHs), normal alkanes, and tetracyclic terpanes, hopanes, and steranes/diasteranes were also studied in dated sediment cores from the Cornwall-Lake St. Francis reach of the St. Lawrence River. The emphasis was to establish a historic record and to follow changes in hydrocarbon inputs to the sediments and where possible, to distinguish among the several likely sources.

COMMUNITY RESPONSES OF MIDGES TO A GRADUAL VERSUS SUDDEN CONTAMINANT EXPOSURES

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Environmental disruptions can be chronic or acute and may produce different biological effects. A 'pulse' is a single, strong, transitory event that imposes an acute shock to the system, analogous to a toxicant spill. A 'press' event is characterised by continuous (chronic) application of (frequently low-level) stress. We studied changes in chironomid abundance and generic richness in 10,000 L microcosms that received either multiple low-concentration or single high-concentration injections of creosote (a complex PAH/hydrocarbon mixture). Chronic application of stress might result in acclimation, ameliorating loss of numbers or richness compared to a single, sudden application of an equivalent amount of toxicant. Six microcosms received semi-daily subsurface injections of creosote to produce a log-series of concentrations ranging from 0.1-30 µg/L after 45 d. Six other microcosms received single creosote injections (0.1-30 µg/L) on the 45th day. Triplicate benthic samples of these were collected ever 1—25 d over 95 d. Toxic effects (±50% reduction in abundance or richness) occurred in microcosms receiving 1-3 µg/L or more creosote. In chronic-application microcosms, abundance declined at lower concentrations/before richness declined. There was no evidence of community acclimation to the gradual application of creosote – abundance and richness effects occurred at 1 µg/L according to both application schedules. Recovery was observed during the latter half of the study. Abundance recovered more quickly than richness. However, abundance and richness were consistently lower in multiple-injection microcosms than in equivalent single-application microcosms. Thus, chronic stress effects are more persistent than acute stress effects at the community level.

Mining and Metals

TRACE METALS CONTAMINATION IN URBAN STREAMS AND STORMWATER DETENTION PONDS

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In 1991, trace metal levels were monitored over a nine month period in two urban creeks in the Hamilton Harbour watershed and in two urban stormwater detention ponds in Guelph, Ontario. Water samples were collected both during dry or non-event periods and immediately after wet weather events. Both water and surficial sediment samples were collected and tested for Cd, Cu, Pb, Hg, Ni and Zn. In almost all cases during wet weather conditions, Canadian Water Quality Guidelines for the protection of freshwater aquatic life were exceeded in water for Pb (>7 mg/L), Cu (>4 mg/L), and Zn (>30 mg/L). Both stormwater ponds accumulated trace metals in sediment to levels above the lowest effect level guideline for the protection and management of aquatic sediment in Ontario, and in the case of Zn (>820 mg/kg), above the severe effect level guideline. These levels of contamination raise serious concerns about the use of these and similar facilities as habitat for biota.

THE EFFECTS OF MINEWATER EFFLUENT ON PHYTOPLANKTON COMMUNITY STRUCTURE

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The effects of minewater effluent release on downstream phytoplankton community structure were examined in a series of northern Saskatchewan lakes. Phytoplankton community structure was documented on a monthly basis during the 1993-94 open water seasons using whole water column samples. Coincident water quality samples were also collected. Phytoplankton richness was low immediately below effluent release, and increased progressively downstream. Multivariate analyses of the phytoplankton community and environmental data matrices illustrated a clear spatial gradient. Ni (primarily) and Fe and/or U concentrations were identified as factors influencing phytoplankton community structure.

UTILIZATION OF METALLOTHIONEIN INDUCTION AND ZEBRA AND QUAGGA MUSSELS AS POTENTIAL BIOMARKER AND *in situ* BIOMONITORS, RESPECTIVELY, OF HEAVY METAL CONTAMINATION WITHIN AQUATIC ECOSYSTEMS

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Metallothioneins (MTs) have been identified in zebra mussels (*Dreissena polymorpha*); but not in quagga mussels (*Dreissena bugensis*). The purpose of this study was to: [1] confirm the existence of MTs in quagga mussels; [2] determine whether or not MT induction is a size-related phenomenon; and [3] examine the utility of using these species as *in situ* biomonitors of heavy metal contamination. Mussels collected from the Black Rock Locks and four sites within the Saint Claire River were categorized by size (6 to 30 mm in 5 mm increments). The hemoglobin-cadmium binding assay was used to assess MT levels in the whole body. Results indicate that quaggas produce MT, but at relatively low levels (4.74 and 9.56 pmoles/mg protein in 6-10 and 25-30 size groups, respectively), as compared to zebra mussels (15.31 and 34.72 pmoles/mg protein in 6-10 mm and 25-30 mm, respectively). Size related effects do occur with larger mussels producing more MT than small mussels. Field studies comparing MT levels in zebra mussels collected from the Saint Claire River and the Black Rock Locks, using the 16-20 and 21-25 mm mussels, showed significantly higher MT levels in mussels of both size groups collected from Black Rock Locks, as compared to the St. Claire River sites. Preliminary results suggest that zebra mussels and the corresponding levels of MTs in their bodies may serve as useful *in situ* biomonitors and biomarkers, respectively, of heavy metal contamination in aquatic ecosystems. (Supported by Hearst and Hughes Undergraduate Research Grants to JMS).

CADMIUM TOXICITY TESTS WITH ANIMALS FROM LAB-CULTURES OF *Hyalella azteca* AND *Gammarus fasciatus*

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Hyalella azteca is frequently used in bioassays. To find out if results which are gained with *H. azteca* are representative for other amphipods we tried to establish a lab-culture of *Gammarus fasciatus*. We conducted toxicity tests and experiments on the uptake and depuration kinetics of cadmium under similar conditions for both species with amphipods of the same age. The attempt to culture *G. fasciatus* quickly showed that the maintenance of this lab-culture requires more time than the maintenance of a *H. azteca* lab-culture. In addition to TetraMin® fish food we fed *G. fasciatus* with brine shrimp and preconditioned alder leafs (*Alnus glutinosa*). We were able to keep the culture of *G. fasciatus* functioning for several months. In addition to a description of the culture method, we will present and discuss the results of cadmium toxicity tests with *G. fasciatus* and *H. azteca* and results of the uptake and depuration kinetics of cadmium in both amphipod species.

THE ACCUMULATION AND DISTRIBUTION OF URANIUM IN LAKE WHITEFISH (*Coregonus clupeaformis*) EXPOSED VIA THE DIET

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U mining and milling activities, particularly prevalent in Saskatchewan Canada, cause U enrichment of impacted aquatic systems. As accumulation occurs mainly in the sediments, resident fish are exposed to U primarily via the diet. To address this issue, adult lake whitefish (*Coregonus clupeaformis*) were fed a commercial diet contaminated with three concentrations of U, 100 mg U/kg, 1000 mg U/kg, and 10000 mg U/kg for 10, 30, and 100 d. Bone, gill, gonad, intestine, kidney, liver, muscle, scales, and skin were analyzed for U. The intestines, bone, and scales accumulated the highest concentrations of U in fish exposed to the moderate and high U concentrations. Of the remaining soft tissues analyzed, kidney, gonads, and liver accumulated the highest concentrations of U. No substantive accumulation occurred in either skin or muscle. Exposure duration-dependent accumulation was evident in bone, scales, kidney, and liver of fish fed the highest U concentration. By day 100, U was detected in the scales of all exposed fish. Furthermore, dose-dependent accumulation was evident as concentrations in this tissue were highly correlated to exposure concentration. The analysis of U in scales, bone, kidney, and intestine is recommended for field biomonitoring programs designed to evaluate the biological availability of U to fish.

ARE ALL DISSOLVED ORGANIC MATTERS EQUALLY PROTECTIVE AGAINST BINDINGS IN FISH GILLS

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We exposed small rainbow trout, *Oncorhynchus mykiss*, 1-5 g, to <1 mM of Pb, Cu, Cd and Ag in synthetic soft water in the presence of 0 to 20 mg $C \times L^{-1}$ of dissolved organic carbon (DOC) isolated from two distinct sources. The two types of DOC had previously been shown to bind benzo(a)pyrene to different degrees. We used the deposition of Pb, Cu, Cd and Ag on the gills of rainbow trout as a biological assay to determine whether DOC source makes a difference to the protective effect of DOC against metal toxicity to fish. If DOC source influences the strength of metal-DOC binding, then the largest differences in binding would show up best with Cd and Ag, which bind to fish gills strongly and bind to DOC relatively weakly. The smallest differences in metal binding to fish gills were predicted to occur with Cu and Pb, which bind very strongly to DOC and bind less strongly to fish gills.

PROTECTIVE EFFECTS OF DISSOLVED ORGANIC CARBON AGAINST PHYSIOLOGICAL DISTURBANCES OF WATERBRONE SILVER ON RAINBOW TROUT

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Adult rainbow trout (*Oncorhynchus mykiss*, ~250 g) were exposed to ~0.1 uM Ag (as $AgNO_3$) in synthetic soft water in the absence or presence of dissolved organic carbon (DOC; ~10 mg $C\text{-}L^{-1}$). The fish were fitted with dorsal aortic cannulae, for repetitive blood sampling to assess the physiological effects of Ag in the presence or absence of DOC. These full-scale physiological experiments were run in a flow-through system. Previously, we showed that ligands which bind metals (such as thiosulphate binding Ag, and DOC binding Cu and Cd) eliminated the respiratory and ionoregulatory

effects of the metals. It is likely that DOC also protects against the physiological and toxicological effects of silver, through reduced binding of silver at the gills of the fish.

LEAD IN SEDIMENT AND ITS RISK TO NESTING FEMALE DUCKS: A RISK ASSESSMENT MODEL

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The risk to mallards (*Anas platyrhynchos*) ingesting Pb contaminated sediments was assessed. Conditions were based on Pb levels in sediment of wetlands associated with Lake Aylmer Quebec (44, 50N; 71,20W) as reported by the Geologic Survey of Canada (1992). Sediment is known to be a significant source of heavy metal contamination for certain waterfowl. However, little work has been done to quantify this risk, particularly during the breeding season when the female is usually restricted in her foraging to an area relatively close to the nest. A computer based model was developed to include parameters for the following; female duck feeding rates, proportion of food intake representing sediment, the level of Pb in the sediment and toxicokinetic information specific to the particular wetland. These computations provided estimates of Pb loading which were then compared to intake levels known to cause neurological harm to mature ducks. Sediment lead levels for eight different wetlands surrounding Lake Aylmer were assessed. Preliminary results indicate that there is an increased risk of neurological disorders to female mallards that choose to breed in the Bullfrog Bay East region of Lake Aylmer. Waterfowl at the other Lake Aylmer wetlands were not considered to be at an increased risk. The significance of this assessment relative to a waterfowl management strategy for Lake Aylmer is discussed.

Pesticides

THE ANALYSIS OF ORGANOPHOSPHOROUS PESTICIDES IN SURFACE WATER USING SOLID-PHASE DISK EXTRACTION AND GC/MS

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Organophosphorous pesticides (OPs) are generally broad spectrum, nonsystemic insecticides and aracicides used for crop protection. Some OPs, such as diazinon and Azinphos-methyl, are used extensively in agriculture and horticulture because they generally have low environmental persistence. It was estimated that over hundred tons of diazinon was used in Ontario in 1993 in controlling agricultural and turf pests. They are also used to control a wide range of insect pests. Substantial amounts of OPs may enter the aquatic environment through surface runoff, drift and leaching from agricultural lands because many OPs have a relatively high water solubility. They are unstable in the environment, however, are subject to hydrolysis, photolysis, and/or biodegradation. Current method based on liquid-liquid extraction and GC with ECD and NPD used by NLET is applied to the qualitative and quantitative analysis of 14 organophosphorus pesticides from 1 litre water samples at ppb level (µg/L). Solid phase extraction technique is faster, more cost-effective and uses less solvent (minimizing solvent exposure and disposal cost). This paper describes the extraction of OPs from natural waters using solid-phase C8 and C18 disk extraction techniques. Identification and quantitation were accomplished using GC/MS technique in EI and ECNI mode. Advantages of using the solid

phase disk extraction technique will be discussed. Also, detection and quantitation of metabolite will be presented.

WATER QUALITY GUIDELINES DEVELOPMENT FOR THE PROTECTION OF FRESHWATER LIFE FOR ANTI-SAPSTAIN FUNGICIDES

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Sapstain is a fungal discolouration that appears on cut lumber, reducing its value. Currently, two of the most widely used anti-sapstain fungicides in Canada are didecyldimethylammonium chloride (DDAC) and 3-iodo-2-propynyl butyl carbamate (IPBC). DDAC and IPBC have had temporary registration for sapstain control in Canada since 1991. In the Fraser River estuary region of British Columbia, lumber mills use more than 500,000 kg of DDAC and IPBC annually. The Fraser River Action Plan is currently monitoring ambient concentrations of DDAC and IPBC in receiving waters and investigating the toxicity of DDAC and IPBC to ecologically relevant species. Despite best management practices, there is a continuing concern with DDAC and IPBC entering aquatic systems via stormwater runoff from lumber yards. The available information on the environmental fate and toxicity of DDAC and IPBC is limited, making an assessment of potential impacts on aquatic ecosystems difficult. Preliminary results of a fate study in the Fraser River indicate that a significant portion of the DDAC present is bound to suspended solids. DDAC and IPBC are moderately water soluble, persistent under some conditions, and moderately toxic to fish and invertebrates. The available 96 h LC_{50} for fish range from 67 to 1900 µg/L for IPBC and from 0.74 to 1300 µg/L for DDAC. No ambient water quality guidelines currently exist for either DDAC or IPBC. Environment Canada has conducted a comprehensive literature search and analysis of the available toxicity data and is proposing ambient water quality guidelines for DDAC and IPBC.

CHEMICAL APPLICATION AS A TREATMENT OPTION TO REDUCE THE RISK OF ACCIDENTAL TRANSFER OF EXOTIC ORGANISMS IN BALLAST WATER

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Nonindigenous organisms, including zebra mussel, ruffe and spiny water flea, have had significant ecological impacts on the Great Lakes aquatic ecosystem. The introduction of these and other exotic species have been attributable to the discharge of ballast water by overseas cargo vessels that enter the Great Lakes. Various physical and mechanical treatments have been considered as measures to reduce the probability of accidental introductions, however, a chemically based approach is one of the few options where current information may allow a treatment process to be developed. Issues that must be considered in its development includes the range of target organisms which can vary from microbes to fish, the toxicological and environmental fate of the chemical, and current regulations on the transport and use of that chemical. The volume of water is a major consideration which can range from 100,000 to more than several million liters. Physical properties of the water can range from fresh- to seawater, temperatures from near zero to >20°C and turbidity can also be a factor. The equipment required and treatment process itself may be an integral part of the vessel itself, or be applied from an external source. There are approximately 1400 transits by large vessels into the Great Lakes annually, about 400 arrive from overseas ports, and the remainder from eastern U.S. and Canadian coastal ports, and ports along St. Lawrence Seaway. There will be negative impacts from the various treatment options considered, nevertheless, these impacts must be weighed against the economic cost of not developing an effective, proactive treatment program.

ENDOCRINE DISRUPTORS IN THE GREAT LAKES BASIN: AN ASSESSMENT OF AGRICULTURAL AND INDUSTRIAL INPUTS

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There appears to be growing evidence that a number of chemicals present in the environment can alter reproductive function mediated by the endocrine system. At least 38 agricultural pesticides and 12 industrial chemicals have been reported to be endocrine disruptors. In Ontario in 1993, the total usage amount of agricultural pesticides suspected of being endocrine disruptors was 1,743,766 kg. This was very similar to the total of 2,131,350 kg of industrial chemicals released to the environment (air, water and land) suspected of being endocrine disruptors. The major compounds include: atrazine, 2,4-D, metribuzin, mancozeb, styrenes and lead. The use and/or release of agricultural pesticides and industrial compounds suspected of being endocrine disruptors will be examined from a Great Lakes Basin perspective.

MONITORING FERTIGATION PRACTICES IN TWO ONTARIO GREENHOUSES

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The long term objective of this study is to address the perception that excessive use of water and fertilizers leads to contamination of local ecosystems. Source water, fertilizer delivery, crop usage and the nutrient content of root zone leachate were monitored over a two year period for two Ontario commercial cut rose growers. The ion content of source water varied with location (grower) and season. Nutrient supply varied with grower but not appreciably with time. Nutrient concentration in the leachate varied between growers and seasons. Nutrients such as ammonium-N were always found to be significantly depleted in the leachate compared to the supply solution. Phosphate, sulphate, sodium and chloride were clearly elevated in the leachate solution indicating accumulation. Other nutrients (nitrate-N, Ca, Mn, K) fluctuated, sometimes accumulating and other times being depleted or unchanged. The accumulation and/or fluctuation of nutrient content in the leachate makes it difficult for growers to routinely re-use the solution. Successful production of greenhouse commodities requires a continuous commitment to quality. Since fertilizer costs are relatively inexpensive, the incentives to reduce runoff from greenhouses are moral obligations to the environment, personal health and legislation.

PERSISTENT ORGANOCHLORINES IN BURBOT FROM THE FRASER RIVER BASIN

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A survey of organochlorine contaminants (OCs) in burbot (*Lota lota*) from 4 lakes within the Fraser River basin was conducted to: [1] evaluate accumulation of OCs, particularly toxaphene at the highest aquatic trophic levels from atmospheric and local sources; [2] compare these data to those being collected in concurrent sediment core studies; and [3] determine if levels might be of a risk to consumers. Toxaphene, in particular, had been identified as a health concern in some lakes in the Yukon. Large (>50 cm) burbot were collected from Stuart, Moose, Kamloops and Nicola Lakes. Liver tissue from each individual analyzed for a spectrum of OC pesticides and PCB congeners, including chlordane, DDT, HCH, toxaphene, non-ortho and coplanar PCBs. Concentrations varied greatly between the four lakes sampled. Measured levels were attributable to both atmospheric transport/deposition and local contamination through historical use of the OC within the lake drainage basin. For example, high DDE in burbot from Nicola Lake is a remnant of historical DDT usage. Of particular interest and concern were results from Moose Lake, where: [1] toxaphene levels exceeded HWC human consumption guideline (100 µg/kg wet weight) by a factor of 2-4x; [2] relatively elevated ΣDDT having a ratio of DDE/DDT suggesting contamination by an unweathered atmospheric source of the insecticide; and [3] PCB congener profiles suggesting a local (as yet unidentified) contamination. Despite upstream effluent discharges, OC levels in Kamloops Lake were, in general, the lowest of the four sampled.

PESTICIDES IN ALBERTA SURFACE WATERS

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INTRODUCTION

Federal and Provincial agencies have collected pesticide residue data from Alberta surface waters since 1971. Much of the sampling effort has focused on fixed locations in major rivers within the National Parks, at provincial and international boundaries, and at key locations above and below major cities. With the exception of 2,4-D, lindane, and ·BHC, which have been detected in at least 20% of these samples, pesticide detections have been infrequent. Generally, reported concentrations have been well below Canadian Water Quality Guidelines (CWQG) for the protection of aquatic life and for irrigation (CCME, 1997). Although the database indicates that pesticides have not affected the quality of water for these sensitive uses, it provides an incomplete picture of pesticides in Alberta surface waters. Few sampling sites were located in intensive agricultural areas and no data exist for small streams and lakes. Furthermore, the standard list of pesticides being monitored was outdated.

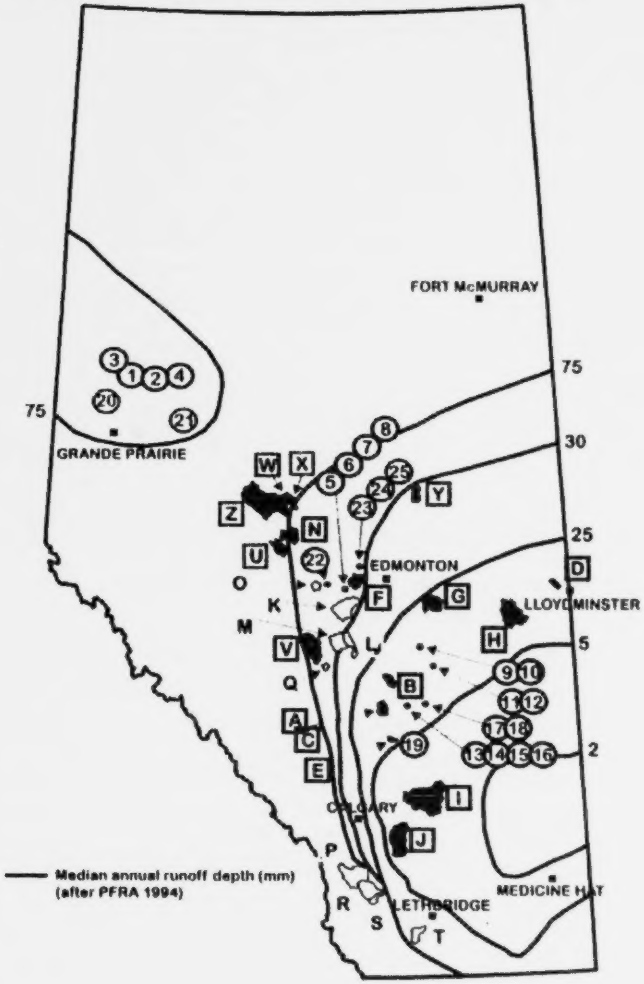
The purpose of this scoping-level study was to determine if agricultural pesticides, sold and used in large quantities across Alberta, were detectable in small streams and lakes and to compare their concentrations with CWQG for the protection of aquatic life and for irrigation. We hypothesized that the pattern of detections would be influenced by the intensity of pesticide use in the drainage basins and by the median annual runoff. Using data from 1995 and 1996, we tested our hypothesis by examining pesticide detections in streams and lakes draining land where pesticide use differs, and by comparing patterns of detections in lakes located in different runoff zones.

METHODS

Site Selection

Census data for pesticide expenditures (Statistics Canada, 1991), incorporated in the Soil Landscapes of Canada database (Shields et al., 1991), were used to rank soil landscape units in Alberta, providing an indication of pesticide use (MacAlpine et al., 1995; Anderson et al., 1996). Units which ranked in the upper quartile were considered to have high pesticide use, those which ranked between the 25th and 75th percentiles were labeled as having medium pesticide use, and those in the lower quartile had low pesticide use (Fig. 1). High use of pesticides occurs mainly in central and southern Alberta (Lloydminster - Edmonton - Calgary - Lethbridge), but high use areas can also be found in north central (north west of Edmonton) and northwestern (Grande Prairie - Peace River) Alberta.

FIGURE 1: Locations of Streams and Lakes


FORT McMURRAY
GRANDE PRAIRIE
EDMONTON
LLOYDMINSTER
CALGARY
LETHBRIDGE
MEDICINE HAT
75
75
30
25
5
2
① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩ ⑪ ⑫ ⑬ ⑭ ⑮ ⑯ ⑰ ⑱ ⑲ ⑳ ㉑ ㉒ ㉓ ㉔ ㉕
A B C D E F G H I J K L M N O P Q R S T U V W X Y Z
Median annual runoff depth (mm)
(after PFRA 1994)

LAKES

HIGH PESTICIDE USE

① BELLOY RESERVOIR
② CODESA LAKE
③ LAKE N.E. RYCROFT
④ LAKE NEAR EAGLESHAM
⑤ LONGHURST LAKE
⑥ H442S1
⑦ H442S2
⑧ H442S3
⑨ H508S1
⑩ H508L1
⑪ H512S1
⑫ WINDSOR LAKE
⑬ GADSBY LAKE
⑭ H515S1
⑮ H515S2
⑯ H515S3
⑰ FOXALL SOUTH
⑱ H537S1
⑲ BRACONNIER RESERVOIR

LOW PESTICIDE USE

⑳ LAKE IN SADDLE HILLS
㉑ DOLLAR LAKE NORTH
㉒ L703S1
㉓ CHICKAKOO LAKE
㉔ L440S1
㉕ MUIR LAKE

STREAMS

HIGH PESTICIDE USE

A RAY CREEK
B HAYNES CREEK
C THREEHILLS CREEK
D STRETTON CREEK
E RENWICK CREEK
F ATIM CREEK
G AMISK CREEK
H BUFFALO CREEK
I CROWFOOT CREEK
J WEST ARROWWOOD CREEK
J ARROWWOOD CREEK

MEDIUM PESTICIDE USE

K STRAWBERRY CREEK
L LLOYD CREEK
M BLINDMAN RIVER
N LITTLE PADDLE RIVER
O TOMAHAWK CREEK
P WILLOW CREEK
Q BLOCK CREEK
R TROUT CREEK
S MEADOW CREEK
T PRAIRIE BLOOD CREEK

LOW PESTICIDE USE

U PADDLE RIVER
V ROSE CREEK
W CHRISTMAS CREEK
X GOOSE CREEK
Y FLAT CREEK
Z SAKWATAMAU RIVER

Sequential searches in the Soil Landscapes of Canada database were used to identify areas in the province which are similar in terms of soil development (chernozemic, luvisolic, and solenetzic), soil texture (fine-grained loam or clay soils) and local topography (typified by long slopes). By selecting streams and lakes in these areas, we ensured that soil landscape features, which can influence contaminant transport in surface runoff, were comparable among drainage basins.

Alberta is typified by a wide range of climatic zones that receive significantly different amounts of runoff (PFRA, 1994). Most areas of central and southern Alberta have very low amounts of runoff (5-20 mm per year); north central Alberta receives 30-75 mm and northwestern Alberta receives more than 75 mm of runoff annually (Fig. 1).

Twenty-seven streams with comparable soil landscape features were chosen in areas of high, medium and low pesticide use. Streams draining the appropriate soil landscape types could not be identified in areas of high runoff; therefore, runoff zones were not incorporated in the study design for streams. A total of 25 small lakes were selected in areas of high and low pesticide use; these lakes could be assigned to zones of high, medium and low runoff (Fig. 1).

Selection of Pesticides for Monitoring

A compilation of pesticide sales records from major agricultural chemical distributors in Alberta for the period 1988 – 1993, providing information for 95 active ingredients, was used to identify pesticides which should be monitored (Cotton and Byrtus, 1995). For this project, 13 compounds, primarily herbicides, (Table 1) were retained for monitoring because the quantities used in agricultural areas of the province were large or because application rates were such that, based on sales records, a large surface area of the province could be treated. Several pesticides meeting these criteria, such as glyphosate and sulfonylurea compounds, were excluded because of analytical costs and limitations. Although many of these pesticides have been monitored in Alberta previously, this is the first time that imazamethabenz has been analyzed in water.

Table 1. List of pesticides retained for monitoring.

Pesticide	Registered use in Alberta
Herbicides	
Bromoxynil	Agricultural
Fenoxaprop-p-ethyl	Agricultural
Diclofop-methyl	Agricultural
Triallate	Agricultural
Ethalfluralin	Agricultural
Imazamethabenz	Agricultural
Trifluralin	Agricultural + domestic (limited)
Dicamba	Agricultural + industrial + domestic + landscape
MCPA	Agricultural + industrial + landscape
2,4-D	Agricultural + industrial + domestic + landscape
Picloram	Industrial + landscape + agricultural (discontinued)
Fungicide	
Carbathiin	Agricultural + municipal (limited)
Insecticide	
Lindane	Agricultural

Sampling Methods

Sampling was carried out during the open water season in 1995 and 1996. Each location was sampled at least twice in 1995: once in early July, then either after a rainstorm during the summer, or in the fall. In 1996, an additional sample was taken from streams during spring runoff and from lakes in June.

Surface grab samples were collected from streams and small lakes, while surface composite samples, consisting of 10 to 15 sub-samples, were collected from larger lakes. The analytical laboratory provided sample bottles that were cleaned to trace organic standards. Sampling equipment was cleaned, then rinsed with hexane and acetone, and wrapped in aluminum foil between sampling sites.

Laboratory Methods

Samples (2 L, unfiltered) were extracted with dichloromethane (DCM and extracts were analyzed by Gas Ghromatography-Mass Spectroscopy using Selected Ion Monitoring (GC/MS – SIM) (Bruns et al., 1991); the method detection limit was 0.02 µg/L (ppb) for most analyses.

Quality Assurance/Quality Control (QA/QC)QA/QA samples (26 in total), either splits, spikes, or blanks, were submitted 'blind' with each batch of samples that was sent to the analytical laboratory. Reported concentrations in 11 pairs of split samples varied by less than 25%. This variability suggests that some compounds may not have been detected (i.e., there is a possibility of false negative detections). The absence of detections in 5 blank samples indicates that the possibility of false positives is low. Twenty-eight spike recoveries from environmental samples were below 100%; recoveries for 4 spikes, in a blank sample, were higher to much higher than 100%. Therefore, actual concentrations in water may be higher than reported (detection frequency and reported concentrations may be biased low rather than high). In light of these QA/QC data, the database for this study is believed to provide a conservative indication of pesticide contamination in Alberta streams and lakes.

Table 2. Pesticide detections in Alberta streams and lakes which drain land receiving HIGH, MEDIUM and LOW quantities of pesticides, and for lakes in different runoff zones (1995, 1996).

	Pesticide use	Runoff zone, mm/yr	No. samples	No. samples with detections	% Samples with detections	Total no. detections	Average no. of detections per sample
Streams (27 sites)							
	HIGH		42	31	73.8%	67	1.6
	MEDIUM		39	9	23.1%	12	0.3
	LOW		29	6	20.7%	8	0.3
	OVERALL		110	46	41.8%	87	0.8
Lakes	(25 sites)						
	HIGH	> 75	22	20	90.9%	34	1.5
		30-75	20	12	60.0%	22	1.1
		5-20	50	19	38.0%	33	0.7
	LOW	> 75	9	6	66.7%	9	1.0
		30-75	22	6	27.3%	7	0.3
	OVERALL		123	63	51.2%	105	0.9

RESULTS

Detection Frequency

At least one pesticide was detected in 44% of the stream samples (n=110) and 51% of the lake samples (n = 123) (Table 2); in many samples, several pesticides were detected (up to 6 per sample). Of the 13 compounds analyzed, 2,4-D, MCPA, imazamethabenz, triallate, dicamba, bromoxynil, and picloram were detected in both lakes and streams (Table 3). Trifluralin and lindane were detected in streams, diclofop-methyl was detected in lakes, but fenoxaprop-p-ethyl, ethalfluralin, and carbathiin were not detected in either lakes or streams. The four most commonly detected compounds overall were 2,4-D, MCPA, imazamethabenz and triallate.

The most notable difference in detections between 1995 and 1996 was an increase in imazamethabenz detection frequency from 3.4% in 1995 to 16.0% in 1996. Imazamethabenz is used mainly on wheat and barley in Alberta; seeded acreage of these two crops increased by about 15% between 1995 and 1996 (AAFRD, 1997).

Influence of Intensity of Pesticide Use

Streams and lakes draining land with high pesticide use had, on average, higher detection frequencies per sample, higher peak concentrations, and a larger number of pesticides detected than those draining basins with low pesticide use (Table 2). Residues of MCPA, bromoxynil, 2,4-D, picloram and triallate were, however, detected in some samples from small lakes in non-agricultural areas (Table 3). In high use areas, frequency of detection, concentration of pesticides, and number of detections were generally highest in zones of high runoff and lowest in zones of low runoff (Table 2).

Compliance with Canadian Surface Water Quality Guidelines

Currently, Canadian Surface Water Quality guidelines for the protection of aquatic life are available for 9 of the 13 pesticides monitored in this study (Table 3). One lindane detection (streams) and one triallate detection (lakes), both in areas of high pesticide use, did not comply with guidelines, but all other recorded concentrations were below guidelines.

There are also Canadian Water Quality Guidelines for irrigation for 4 of the 13 pesticides monitored. These guidelines are more restrictive than those for the protection of aquatic life and were therefore exceeded more frequently. All detections of dicamba in both streams and lakes exceeded the irrigation guideline, which is below the method detection limit used in this study. Most detectable concentrations of MCPA also exceeded the irrigation guideline, as did some of the bromoxynil detections. Most incidences of non-compliance in lakes and streams occurred in areas of high pesticide use.

DISCUSSION and CONCLUSIONS

The results of pesticide monitoring conducted on a selection of streams and small lakes across Alberta confirm that several pesticides currently used in the province occur at measurable concentrations in surface waters. There are at least 96 active ingredients on the Alberta market and well over 40 of these are in common use. Monitoring of 13 of these compounds can only provide a scoping-level picture of the incidence of pesticide contamination in the province's surface waters.

Of the nine compounds detected in this survey, all except imazamethabenz and diclofop-methyl had been detected previously in large Alberta rivers (Anderson, 1995). Detection frequency in this study was higher than reported previously. However, lindane was detected considerably more frequently in past and usually at concentrations well below the method detection limit for this study. Most compounds detected in this study have also been found in surface waters in Manitoba (Currie and

Table 3. Maximum Pesticide Concentrations (µg/L) and Noncompliance with Canadian Water Quality Guidelines-CWQG (CCME 1997) for the Protection of Aquatic Life (PAL) and for Irrigation (IRR) in Alberta Streams and Lakes which Drain Land Receiving HIGH, MEDIUM and LOW Quantities of Pesticides, and for Lakes in Different Runoff Zones (1995 & 1996).

		Dicamba	MCPA	Bromo-xynil	Diclofop methyl	2,4-D	Picloram	Trifluralin	Triallate	Lindane	Ethal-fluralin	Carba-thiin	Fenoxa-prop-p-ethyl	Imaza-metha-benz
IRR (µg/L)		*0.006*	*0.03*	*0.35*	*0.18*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
PAL (µg/L)		*10*	*2.6*	*5*	*6.1*	*4*	*29*	*0.1*	*0.24*	*0.01*	*NA*	*NA*	*NA*	*NA*
27 STREAMS (110 samples)														
						Maximum Concentration (µg/L)								
HIGH PESTICIDE USE		0.08	0.55	0.71	-	0.46	0.65	0.047	0.16	0.051	-	-	-	1.4
MEDIUM PESTICIDE USE		0.092	0.022	-	-	2.1	-	-	0.033	-	-	-	-	0.12
LOW PESTICIDE USE		-	0.026	0.022	-	1.2	0.2	-	0.076	-	-	-	-	-
						Number of Noncompliant Concentrations/Total Detections								
Guideline Exceeded		*IRR*	*IRR*	*IRR*						*PAL*				
HIGH PESTICIDE USE		4/4	11/13	1/5	NA/0	0/23	0/4	0/2	0/7	1/1	NA/0	NA/0	NA/0	NA/16
MEDIUM PESTICIDE USE		1/1	0/3	0	NA/0	0/6	0/0	0/0	0/1	0/0	NA/0	NA/0	NA/0	NA/1
LOW PESTICIDE USE		0	0/1	0/1	NA/0	0/3	0/1	0/0	0/2	0/0	NA/0	NA/0	NA/0	NA/0
25 LAKES (123 samples)														
						Maximum Concentration (µg/L)								
	Median Annual Runoff													
HIGH PESTICIDE USE	> 75 mm	4.6	1.7	-	-	-	-	-	0.022	-	-	-	-	2.3
	30 - 75 mm	-	0.2	0.39	0.036	0.34	-	-	0.48	-	-	-	-	-
	5 - 20 mm	0.07	0.39	0.39	0.036	0.36	-	-	0.48	-	-	-	-	0.44
LOW PESTICIDE USE	> 75 mm	-	0.039	0.11	-	0.14	0.029	-	0.021	-	-	-	-	-
	30 - 75 mm	-	0.021	-	-	0.073	-	-	0.052	-	-	-	-	-
						Number of Noncompliant Concentrations/Total Detections								
Guideline Exceeded		*IRR*	*IRR*	*IRR*					*PAL*					
HIGH PESTICIDE USE	>75 mm	6/6	6/6	0/0	0/0	0/0	0/0	0	0/4	NA/0	NA/0	NA/0	NA/0	NA/8
	30-75 mm	0/0	5/5	1/3	0/1	0/5	0/0	0	1/3	NA/0	NA/0	NA/0	NA/0	NA/5
	5-20 mm	2/2	13/13	0/0	0/1	0/16	0/0	0	0/2	NA/0	NA/0	NA/0	NA/0	NA/0
LOW PESTICIDE USE	> 75 mm	0	1/1	0/1	0/0	0/5	0/1	0	0/1	NA/0	NA/0	NA/0	NA/0	NA/0
	30 - 75 mm	0	0/1	0/0	0/0	0/3	0/0	0	0/3	NA/0	NA/0	NA/0	NA/0	NA/0

NA = no guideline available
- = no detections

Williamson, 1997) and in Saskatchewan lakes and dugouts (Donald and Syrgiannis, 1995; Grover and Cessna, 1996).

Comparing concentration patterns in small and large streams, Wauchope et al. (1994) describe short pulses of high pesticide concentrations in small streams and longer pulses of low pesticide concentrations in larger streams as being most typical. However, the maximum concentrations reported in this study tend to be lower than those reported for large streams in the long-term database. Several factors related to sampling frequency and climate can account for this situation. Times of peak pesticide concentrations could have easily been missed as a result of the low sampling frequency and the short duration of our sampling program. Furthermore, in 1995 and 1996, heavy rains causing runoff did not occur after the main period of herbicide application in drainage basins where pesticide use is most intense.

Although several pesticides monitored in this study have industrial, municipal, and domestic uses in addition to agricultural applications, our results indicate that there is a positive relationship between the intensity of agricultural pesticide use and the level of pesticide contamination of surface waters, within a drainage basin. Detection frequency, concentration, and non-compliance with surface water quality guidelines tend to be higher in areas of high pesticide use than in areas of low pesticide use. However, local agricultural use patterns do not explain all occurrences of pesticides in this study. In one instance, roadside spraying was identified as the most likely reason for a picloram detection in a small, remote lake in northwestern Alberta. In other instances, there was no clear explanation for the presence of pesticides. Detections in lakes and streams draining land with little or no use of pesticides suggests that atmospheric transport and deposition may be contributing to the presence of pesticides in Alberta surface waters. Such pathways of pesticide entry to water bodies have been documented in other parts of Canada (Muir and Grift, 1995; Waite et al., 1995).

Higher frequencies of pesticide detections have been reported in surface waters after rain events (e.g., Senseman et al., 1997) and especially when rain occur shortly after pesticide application (e.g., Wauchope et al., 1994). Our results also indicate that for a given level of pesticide use, regional differences in pesticide detections may be related to annual runoff, and therefore to the amount of precipitation. Consequently, streams and lakes in high runoff zones may have a higher risk of becoming contaminated than surface waters in low runoff zones.

Canadian Water Quality Guidelines have yet not been determined for several of the pesticides monitored in this study. Where guidelines exist, compliance was high with guidelines for the protection of aquatic life, but was low with irrigation guidelines. These results suggest that herbicide use could have detrimental effects on sensitive, irrigated crops, although in this study, few of the streams and none of the lakes monitored supply irrigation water.

ACKNOWLEDGEMENTS

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TOXICITY OF THE SYNTHETIC SLOW RELEASE FERTILIZER, NPK (7-40-0), ON VARIOUS FRESHWATER ORGANISMS

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The synthetic slow release fertilizer NPK (7-40-0) is intended to be used as a nutritional enhancement for salmon bearing streams. The toxicity effects of selected freshwater species was investigated. Short term (lethal and sublethal) toxicity tests were performed on aqueous extracts of the fertilizer using standard procedures for juvenile and embryonic rainbow trout (*Oncorhynchus mykiss*), daphnids (*Ceriodaphnia dubia* and *Daphnia magna*) and bacteria (*Vibrio fischeri*). Overlying water spiked with aqueous extracts of the fertilizer was used in 10 d *Hyalella azteca* and *Chironomus tentans* sediment toxicity tests. Preliminary results indicated that complete solubility was difficult to obtain due to the slow release nature of the fertilizer (500 mg/L of dissolved fertilizer was recovered from a 1 g/L fertilizer solution). Ammonia, an active component of the fertilizer, was measured as an indicator of NPK (7-40-0) concentration. Ammonia values were reported as total ammonia (mg/L NH_3). The freshwater amphipod, *H. azteca*, was the most sensitive of the species tested with adverse effects observed at ammonia levels of 8 mg/L NH_3. To lesser extent *O. mykiss*, *C. dubia*, *C. tentans* and *V. fischeri* were adversely effected at ammonia levels of 32 mg/L NH_3.

LETHALITY OF THE ANTI-SEA LOUSE FORMULATION EXCIS® TO LARVAL STAGES OF THE AMERICAN LOBSTER (*Homarus americanus*)

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The pesticide formulation EXCIS® is currently registered for use to combat sea lice infestations on salmonid aquaculture sites in the State of Maine. Its efficacy and effects on non-target oraganisms is being reviewed by the Pest Management Regulatory Agency (Health Canada) for possible registration in Canada. The active ingredient in this formulation is the synthetic pyrethroid, cypermethrin. Lobsters are fished commercially in the vicinity of aquaculture grow-out sites and may be exposed to this chemical during and after de-lousing treatments. The recommended treatment concentration is 5 µg/L. The 48 h LC_{50} of this formulation to the three larval stages (I, II, and III) of the american lobster (*Homarus americanus*) and to the first post-larval stage (IV) were determined. Initial analysis of our results show considerable variability in response but no significant difference in sensitivity between larval stages. The 48 h LC_{50}'s, reported as cypermethrin, were: Stage I = 0.29 µg/L, Stage II = 0.12 µg/L, Stage III = 0.06 µg/L, and Stage IV = 0.12 µg/L. The formulation is lethal to larval lobsters over 48 h at 3% of the recommended treatment concentration. When similar experiments were conducted with pyrethrum extract, a pesticide formally used in sea lice treatments, we found significant differences in sensitivity between larval stages.

DURATION OF EXPOSURE AFFECTS DELAYED TOXICITY OF DIFLUBENZURON TO GRASS SHRIMP EMBRYOS

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ABSTRACT

When embryos of the grass shrimp *Palaemonetes pugio* were exposed for 4 d to single pulse sublethal concentrations of diflubenzuron (DFB), various delayed toxic effects were produced in the larvae. These delayed effects included: Larval viability, terata (larval abnormalities) and duration of larval development (Wilson, 1985). Exposing the embryos for different lengths of time to single test concentration (2.5 μg/L) of DFB affected all parameters measured. The standard length of first stage larvae exhibited negative linear correlation with duration of embryonic exposure. However, the 6th abdominal segment (which is the main organ of propulsion) was the most adversely affected. Measurements of the length and width of this segment showed that the length decreased as the duration of exposure increased but asymptotes at about 2.5 d. Similarly, the width was most significantly affected by exposure duration of between 2 and 5 d. Abnormality indices representing the degree of stuntedness and degree of ballooning also increased with duration of exposure and asymptotes at 4 d. Larval viability exhibited significant negative correlation with duration of exposure (equation: $Y = 74.57 - 17.24X$, where Y is percent survival and X the duration of embryonic exposure). Larval developmental time decreased with exposure duration, with a significant reduction observed at 3 and 4 d exposure. The significance of these findings in the development of the grass shrimp embryo-larval toxicity (GSELTOX) test is discussed.

INTRODUCTION

The larvae, juvenile and adult stages of the grass shrimp have been used extensively in toxicity testing (Buikema et al., 1980; Weber et al., 1996). The vast majority of these tests are acute toxicity tests. Sublethal toxicity tests with these life stages have been few and until the work by Wilson (1985), toxicity tests using grass shrimp embryos was virtually non-existent. The delayed sublethal bioassay (DSB) described by Wilson (1985) was recently modified and proposed as the grass shrimp embryo-larval toxicity (GSELTOX) test (Wilson, 1997). This test essentially involves exposing embryos to single pulse test concentrations and monitoring delayed effects on the larvae. In recent years, interest in the use of grass shrimp embryos in toxicity tests has grown. The sensitivity of these embryos to various toxicants has been amply documented (See Wilson, 1985; Fisher and Foss, 1993; Wilson et al., 1995; Mearns et al., 1995). Similarly, the use of grass shrimp embryos in sediment toxicity studies has been demonstrated by Wilson et al. (1995). Monitoring teratogenesis in the larvae after exposure of the embryos was shown to be more sensitive than chronic exposure of the grass shrimp or crab (*Rhithropanopens harrisii*) larvae.

In a review of sublethal effects of pollutants an fish eggs and larvae, Von Westernhagen (1988) reported that the exposure time and concentration influence the severity of abnormalities. Also the effects of metals on the embryos of the yellow crab (*Cancer anthonyi*) increased as a function of exposure duration (Macdonald et al., 1988). Except for concentration, the duration and frequency of exposure are the most significant factors influencing the toxicity of a chemical (Rand et al., 1995). The present study was conducted to determine the minimum duration of exposure of embryos to single pulse concentration of diflubenzuron that will result in significant delayed toxicity in the larvae.

MATERIALS AND METHODS

Ovigerous female grass shrimp *Palaemonetes pugio* carrying stage 4 embryos (6 d old) were selected from a population that had been laboratory—acclimated for at least 4 d. Five shrimp were introduced into 20 cm diameter Carolina culture bowls containing 1L of either 2.5 μg/L diflubenzuron (DFB) or filtered (to 45 μm) seawater. Shrimp were fed newly hatched *Artemia salina* nauplii (cyst from Great Salt Lake region). Five replicates were set up. The solutions were not renewed, but the animals were fed daily. There was no aeration. The bowls were kept in an environmental chamber set at 25°C and

12:12 L:D cycle. Three ovigerous females were removed daily for the next 7 d from both DFB and seawater control bowls and transferred to freshly prepared seawater. Once shrimp have been transferred, the seawater was changed daily until 1 or 2 d prior to hatching of eggs, when the shrimp were separated and held individually in 9-cm Carolina culture bowls containing 200 ml filtered seawater. The salinity of seawater used throughout this test was 20 PPT, diluted from high salinity natural seawater. After hatching some larvae were examined for morphological abnormalities; others were measured to quantify the abnormalities using abnormality indices (see Wilson, 1985; and Wilson, et al., 1995) as follows: Degree of Stuntedness: $DS = (1-LT_d \div LT_W)\ 100$; where LT_d is length of telson for exposed larvae and LT_W is length of telson for unexposed larvae. The Degree of Ballooning: $DB = (WT_d - WT_W)\ 100$; where WT_d is the telson width for exposed larvae and WT_W the width of telson for unexposed larvae. The rest of the larvae were reared to the postlarval stage using 10 larvae per 7-cm Carolina culture bowl with daily changes of seawater. Fifty larvae were reared from each female. Percent survival to post larvae and duration of larval development were determined. The experiment was replicated with minimum of 3 gravid females per duration per replicate. Regression analysis and analysis of variance (ANOVA) were performed on the data as appropriate (Sokal and Rohlf, 1981). The level of significance was set at $p=0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Exposing grass shrimp embryos to single pulse sublethal concentrations of diflubenzuron (DFB) results in various delayed toxic effects at the larval stage. These delayed effects include: survival to the postlarval stage, teratogenesis (larval abnormalities) duration of larval development, altered phototaxis and decreased swimming speeds (Wilson, 1985; Wilson et al., 1985; Wilson et al., 1987). These effects have been shown to exhibit strong concentration-response relationships. Exposing the embryos for different lengths of time to a single test concentration (2.5 µg/L) of DFB affected all parameters measured at the larval stage. The standard length of 1 d old larvae exhibited negative linear correlation with duration of embryonic exposure (Fig. 1). The regression equation for this correlation is $Y = 2.703 - 0.091\ X$, where Y is the standard length (mm) and X the duration of exposure (d); $r = -0.982$ (significant, $p<0.01$). Despite this correlation, different parts of the body of the larvae were affected differently. The sixth abdominal segment (which is the main organ of propulsion) was

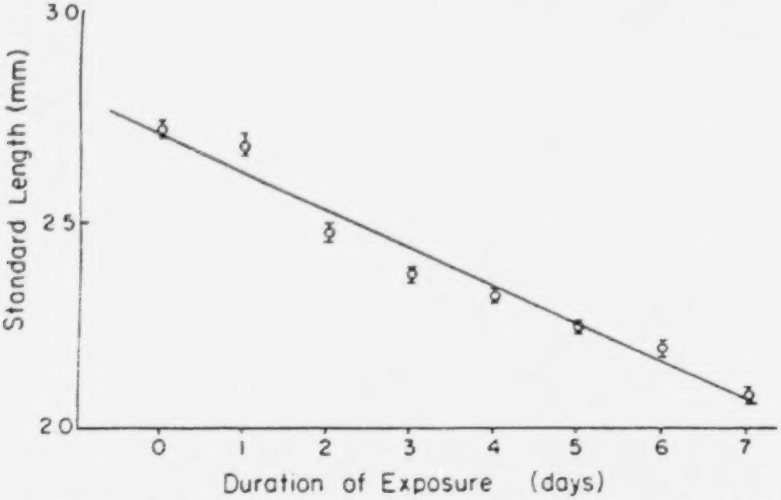

Fig. 1. Relationship between standard length of Stage 1 larvae of *P. pugio* and the duration of embryonic exposure.

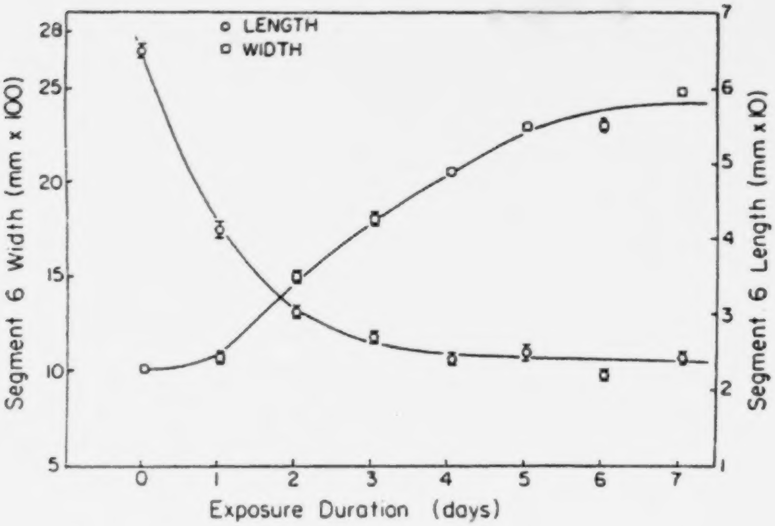

Fig. 2. The length and width of segment six of Stage 1 larvae of *P. pugio* as a function of the exposure duration.

the most adversely affected. The length of this segment decreased with exposure duration up to about 3 d then remains virtually constant (Fig. 2). Also, the width of the 6th abdominal segment increased with duration of exposure up to 5 d. After 5 d, the width of segment 6 does not increase significantly with increase in duration of exposure.

Larval viability exhibited negative correlation with exposure duration (Fig. 3). The equation for this correlation is $Y = 74.57 - 17.4X$; where Y is percent survival and X duration of embryonic exposure; $r^2 = -0.899$ (significant, $p<0.01$). From this equation, it is evident that there is a decrease of 17.24% in larval viability for every day the embryos were exposed to 2.5 µg/L DFB. The calculated abnormality indices for 1 d old larvae hatched from DFB-exposed embryos is shown in Table 1. Both the degree of stuntedness and degree of ballooning increased with exposure duration until an asymptote is reached on day 4 and 5 respectively. This response pattern is similar to the concentration-response

Table 1. Calculated abnormality indices for 1 d old larvae hatched from embryos exposed for different duration to 2.5 µg/L DFB.

Exposure duration, days	Degree of stuntedness, %	Degree of ballooning, %
Control	0	0
1	37	1
2	52	5
3	56	8
4	59	11
5	59	13
6	63	13
7	59	14

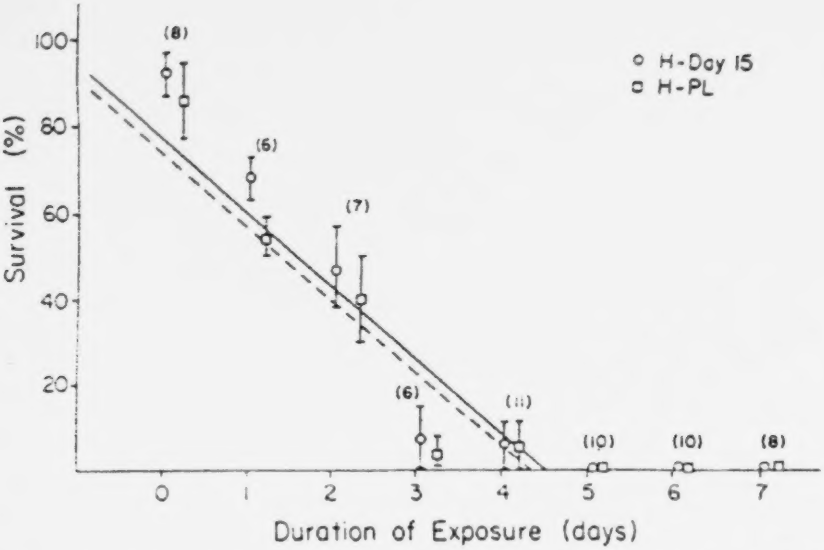

Fig. 3. Effect of duration of embryonic exposure to 2.5 µg/L DFB on the larval development of *P. pugio*. (Mean ± S.D.)

relationship reported for both of these abnormality indices (Wilson, 1985; Wilson, 1997). Similarly, the effects of metals on mortality of embryos of *C. anthonyi* have been shown to increase as a function of exposure duration (Macdonald et al., 1988).

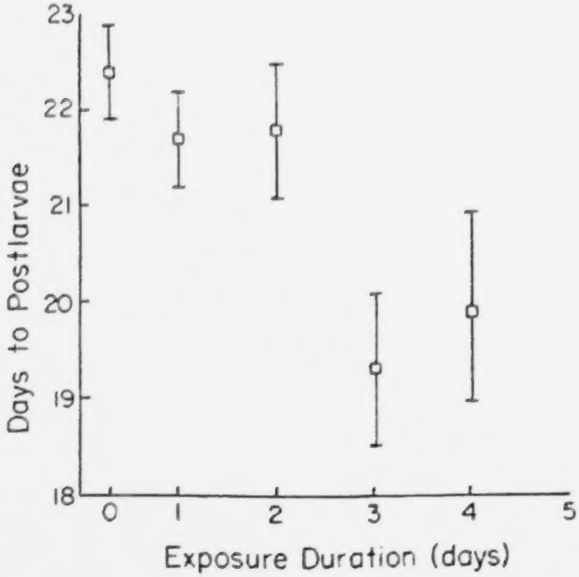

Fig. 4. Relationship between duration of exposure and duration of larval development. (Mean ± S.D.)

The relationship between duration of embryonic exposure to 2.5 µg/L DFB and larval development time is presented in Fig. 4. It is observed that larval development time is shorter for 3 and 4 d exposure than for 1 and 2 d. Similar results have been obtained when embryos of different ages were exposed to different concentrations of DFB (Wilson 1985, 1997). The decrease in larval development time exhibited as a result of increased exposure duration of embryos or increase in concentration of the chemical cannot be fully explained at this time. It is worth noting however, that larvae reared from embryos that have been exposed to 2.5 µg/L DFB molted more often than the unexposed larvae (personal observation). From the foregoing discussion it is apparent that the longer the exposure duration, the more severe the delayed effects.

These results indicate that for grass shrimp embryos exposed to DFB, the minimum exposure duration that result in significant delayed effects that correlate with the survival of the larvae is about 4 d. Therefore, exposing embryos for longer than 4 d is unnecessary as the magnitude of the delayed effects is about the same up to 7 d exposure. This fact is critical for the development of a short term predictive toxicity test using grass shrimp embryos. These findings also support the use of 4 d single pulse exposure by Wilson (1985, 1997) for the grass shrimp embryo-larval toxicity (GSELTOX) test. This study should be repeated with other reference toxicants as the GSELTOX test is being developed.

In conclusion, the severity of the delayed toxic effects of DFB on grass shrimp embryos is dependent on the duration of exposure up to a threshold of 4-5 d depending on the toxicity endpoint measured. Therefore, 4 d single pulse exposure of grass shrimp embryos to sublethal concentrations of pesticide is sufficient to produce significant delayed effects in the larvae.

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Risk Assessment

SOIL HEALTH INDEX FOR CONTAMINATED SITE ASSESSMENT AND REMEDIATION

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Soil health is defined in terms of abiotic and biotic properties and how these relate to current conditions and future potential. Abiotic factors include chemical and physical conditions such as soil pH and electrical conductance (salts), organic matter content, particle size distribution (sand, silt, clay), colour and odour. The spatial plane defined by the abiotic characteristics delineates the area which can support soil life forms and, potentially, a viable soil ecosystem. Biotic factors include an assessment of indigenous bacterial and fungal populations, measurement of soil respiration, and assessment of the potential to support growth of microbes, plants (algae and higher plants), and invertebrates. Collectively, the biotic and abiotic measurements provide an index for soil health which can be applied to assess existing conditions and future potential. The utility of the soil health index for contaminated site assessment and remediation is presented here through a case studies.

EXPLORING APPROACHES FOR THE DERIVATION OF AQUATIC LIFE GUIDELINE/CRITERIA

P.-Y. Caux. Guidelines Division, Environment Canada, Hull, QC.

A common approach to aquatic life guideline/criteria development is to obtain the most sensitive lowest-observable-effects level (LOEL) from a chronic exposure study on a native Canadian species and to multiply it by a safety factor of 0.1. The use of NOELs and LOELs as summary statistics for low toxic effects is presently under scientific scrutiny. In this poster, we explore an additional approach, the EC_x or curve-fitting approach for incorporation into guideline/criteria development. It is proposed that [1] a range of models be available to choose from (e.g., models in the logistic family, probit model, Weibull model) for both continuous and discrete data; [2] the model with the best fit be chosen to derive low toxic threshold levels; [3] EC_x estimates, often model dependent, not be extrapolated beyond the actual toxicity data and, [4] when appropriate, an EC_x of 20% be chosen to replace the LOEL from a chronic exposure study. The EC_x approach does not produce adequate model fits unless there is a clear dose-response relationship and treatments with partial effects. Many current data sets from both the 'grey' and open literature will not necessarily conform to these requirements. Therefore the EC_x approach could be incorporated into Canadian protocols as an additional method in the derivation of aquatic life guideline/criteria.

ENVIRONMENTAL ASSESSMENT OF THE PRIORITY SUBSTANCE ACETALDEHYDE

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As acetaldehyde is currently on the CEPA Priority Substances List, the environmental risks associated with its release into the Canadian environment are being assessed. Over 4,000 tonnes per year of acetaldehyde are released from combustion and other anthropogenic sources, with large though unquantified amounts being released from natural sources and through oxidation of organics in air. Since highest environmental concentrations are associated with vehicle and industrial emissions, the assessment will focus on possible effects to terrestrial biota close to vehicle and industrial emissions, and aquatic biota near industrial effluents.

IS THE CEPA PRIORITY SUBSTANCE PHENOL A CONCERN TO THE CANADIAN ENVIRONMENT? AN ECOLOGICAL RISK ASSESSMENT

T. Lugsdin and R. Breton. Commercial Chemicals Evaluation Branch, Environment Canada, Hull, QC.

In Canada, substances on the Priority Substances List (PSL) are assessed by two federal government departments, Environment Canada and Health Canada, to determine if they pose a risk to the environment and to human health. This presentation will discuss the ecological risk assessment only. On December 16, 1995, 25 substances were added to the PSL, including phenol. After extensive collection of data, data gaps were identified, including information on entry/sources and exposure of phenol to the Canadian environment. Several mechanisms were set up to fill the data gaps including the establishment of an environmental resource group and the mandatory requirement for industry to provide data. The assessment focuses on specific key industrial source sectors, considering the types of releases of phenol to the Canadian environment, the fate and effects of phenol and the ecological risk analysis approach. The methods used to conduct the assessment are provided with a rationale for the selection of the assessment and measurement endpoints.

ENVIRONMENTAL RISK ASSESSMENT OF THE PRIORITY SUBSTANCE ACROLEIN UNDER THE CANADIAN ENVIRONMENTAL PROTECTION ACT (CEPA)

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Acrolein (C_3H_40) is not commercially produced in Canada and its only identified non-pesticidal use is as a hydrogen sulphide scavenger in oil drilling operations. Although acrolein is registered in Canada for use as an aquatic herbicide and slimicide, this assessment will focus on non-pesticidal entry, exposure and effects of acrolein in the Canadian environment. A comprehensive review of the available literature and industry surveys revealed no detectable concentrations (<0.1 μg/L) for acrolein in surface waters. Air emissions to the environment can be from natural sources, such as forest fires, whereas anthropogenic sources include vehicle exhaust and emissions from pulp and paper plants. Concentrations for acrolein in air ranged from below detection (0.0005 mg/m^3) to a maximum of 0.00485 mg/m^3. Few or no data are available for concentrations of acrolein in soil, sediment and groundwater. There are extensive acute effects data for aquatic and terrestrial organisms while chronic effects data were only located for mammalian laboratory animals. A Tier 1 (hyperconservative) analysis found that there is potential for environmental effects on the most sensitive terrestrial and aquatic organisms reported in the literature.

THE IMPACT OF TRIFLUOROACETIC ACID ON MODEL AQUATIC ECOSYSTEMS

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Trifluoroacetic acid (TFA) is the atmospheric degradation product of a number of hydrofluorocarbon and hydrochlorofluorocarbon coolants that are being introduced to replace ozone-depleting chlorofluorocarbon counterparts. The production and use of these new compounds is expected to increase ten-fold over the next decade. TFA is highly water soluble and very stable in the environment; therefore, it remains in the water column for extended periods of time. A study was undertaken at the Guelph Microcosm Facility during the summer of 1997 to assess the impact of TFA on aquatic ecosystems. Ponds were treated at 10, 100, 300 and 1000 μg/L with the two lowest concentrations having four replicates and the two highest being dosed in duplicate. The impact upon periphyton communities was monitored through ^{14}C assimilation, ash-free dry weight analysis, chlorophyll content, biomass and species diversity. Phytoplankton communities were monitored through ^{14}C assimilation, biomass and species diversity. TFA is believed to inhibit the citric acid cycle through the formation of either di- or monofluorocitrate, resulting in elevated concentrations of citric acid in tissue and serum. The levels of citric acid were monitored in the plant *Myriophyllum spicatum* and in the fish *Pimephales promelas* to determine if TFA was having any impact upon the function of the citric acid cycle. The results of these experiments will be discussed.

A COMPARISON OF TWO MIXING MODELS FOR THE RISK ASSESSMENT OF AMMONIA FROM SEWAGE EFFLUENTS

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Environment Canada is conducting an ecological risk assessment of ammonia in the aquatic environment under the Priority Substances List II program. The estimated total ammonia load to aquatic ecosystems from Municipal Sewage Treatment Plants, based on 25 mg/L concentration in sewage and Environment Canada data on sewage flow rates, is 112,000 tonnes/year. While many studies have assessed the impacts of sewage effluents on stream biota, toxicity data for the ammonia component is limited. Determining the 'worst case scenario' concentration of ammonia in a dispersing sewage plume will be the first step in evaluating the potential impact of the ammonia component. Due to data availability, the South Saskatchewan river at the Saskatoon WWTP (waste water treatment plant) has been chosen as the model evaluation site. Two hydrodynamic mixing models, WASP and CORMIX, are being evaluated for their applicability to this assessment. CORMIX was designed for the analysis, prediction, and design of aqueous discharges into watercourses, with emphasis on the geometry and dilution characteristics of the initial mixing zone. WASP is a more complex model, and contains a eutrophication subroutine that predicts nitrogen dynamics. Calibration and validation are necessary for accurate modeling, and will be conducted on the selected model. Data collected by the National Hydrology Research Centre and University of Alberta for the study 'Nutrient Dynamics in Riverbeds: the Impact of Sewage Effluent and Aquatic Macrophytes' is considered in this assessment. Concentrations of ammonia in the effluent, flow rates of the river, and cross-sectional data have been obtained from the City of Saskatoon, NHRC and the Province of Saskatchewan.

PROBABILISTIC RISK ASSESSMENT OF PESTICIDE RESIDUES FOUND IN SURFACE WATERS FROM THE CANADIAN SECTION OF THE GREAT LAKES BASIN

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Because of the intensity and close proximity of agricultural practices to various aquatic systems, residues of many pesticides have been found in the surface waters of the Great Lakes Basin. Triazine herbicides, organophosphorus insecticides, organochlorine pesticides, phenoxy herbicides, and dinitroaniline herbicides have all been detected in various surface waters of Ontario. A probabilistic risk assessment technique was used to assess the risks associated with the exposure of aquatic organisms (fish, invertebrates, and plants) to these agricultural pesticides. The use of probabilistic approaches to higher tiers of risk characterization offers advantages over the use of deterministic approaches (i.e. Water Quality Guidelines for the Protection of Aquatic Life) which are based on 'single point' toxicity 'values' from the most sensitive species and worst-case exposure 'scenarios' to the stressor. These advantages include the use of all data (described as a distribution) for both effects and exposures and a tendency toward greater stability of the resulting characterization with increasing availability of data. In most Great Lakes Basin cases, exposure concentrations did not exceed the effects distribution at the 10th centile assessment criterion, indicating low risks to aquatic organisms exposed to these concentrations. However, the loading of multiple organophosphorus insecticides and in particular, diazinon, may pose a significant risk to aquatic organisms at specific Great Lakes Basin sites.

THE FIRST STEP IN COASTAL MANAGEMENT: MARINE ENVIRONMENTAL ASSESSMENT. AN EXAMPLE FROM THE GULF OF ST. LAWRENCE AND ESTUARY

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There is an emerging trend world-wide for ecologically-based management of coastal systems for solving environmental problems. Using ecological principles and concepts requires that science be the basis for providing a common understanding of ecosystem processes and attributes. A marine environmental assessment describes these processes and attributes in a manner that is understandable to all participants in integrated management: managers, public, community and resource groups, industry and even other scientists. The Marine Environmental Assessment of the Estuary and Gulf of St. Lawrence is a multidisciplinary summary of the accumulated scientific understanding of the this marine region. It points out principal uncertainties that hinder our understanding of the marine environment, the extent of anthropogenic modifications to this marine system, and identifies important regional environmental issues. We describe the oceanography of the system to provide an understanding of the general physical characteristics to which all organisms must adjust. The biological characteristics of the system describe the living aspects of the region. Fisheries resources are discussed in more detail than other biological organisms because of their commercial importance. The sources and distributions of chemicals, both natural and anthropogenic in origin, are described. Throughout, anthropogenic modifications are highlighted. The final chapter identifies and assesses the most important issues involving contaminants and anthropogenic modifications to the environment. Recommendations are made for research that are required to resolve uncertainties in scientific knowledge.

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BEST STUDENT PAPER AWARDS/PRIX POUR LES MEILLEURS EXPOSÉS PAR DES ÉTUDIANTS

BEST PLATFORM PRESENTATION

An evaluation of the cytotoxicity and photocytotoxicity of intact and photomodified creosote through the use of a rainbow trout gill cell line, TRgill-W1, and two fluorescent indicator dyes, alamar Blue and 5-carboxyfluorescein diacetateacetomethylester.

Kristin Schirmer

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BEST POSTER PRESENTATION

Lead in sediment and its risk to nesting female ducks: a risk assessment model

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The Proceedings of each Annual Aquatic Toxicity Workshop have been published in a series of Technical Reports listed below. These Proceedings are generally provided to each Workshop participant, and are also sent to selected libraries, government departments and other agencies. Copies of 4th and subsequent Proceedings may be available for a charge, as photocopies or fiche, from Micromedia Limited, 240 Catherine Street, Suite 305, Ottawa, ON, K2P 2G8 (613-237-4250).

Proceedings of the 23rd Annual Aquatic Toxicity Workshop: October 7-9, 1996, Calgary, Alberta. Edited by J.S. Goudey, S.M. Swanson, M.D. Treissman and A.J. Niimi. Can. Tech. Rep. Fish. Aquat. Sci. 2144: 196 p.

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